SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVAULATION OF SOME NEWER SERIES OF QUINAZOLONES

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University,

Chennai- 600032.

In the partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

Submitted by

SANKARNARAYANAN. S

Reg.No. 26106035

Under the Guidance of

Mr. A. THIRUGNANA SAMBANTHAN, M.Pharm., (Ph.D.)

Assistant Professor,

Department of Pharmaceutical Chemistry,

ADHIPARASAKTHI COLLEGE OF PHARMACY,

(Accredited By "NAAC" with CGPA of 2.74 on a Four point Scale at "B" Grade)

MELMARUVATHUR- 603319.

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CERTIFICATE

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Place: Melmaruvathur

Date:

Mr. A. THIRUGNANA SAMBANTHAN, M.Pharm., (Ph.D.)
Assistant Professor,
Department of Pharmaceutical Chemistry,
Adhiparasakthi College of Pharmacy,
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CERTIFICATE

This is to certify that the dissertation entitled **"SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVAULATION OF SOME NEWER SERIES OF QUINAZOLONES"** is the bonafide research work carried out by **SANKARNARAYANAN. S** in the Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamilnadu Dr. M.G.R Medical University under the guidance of **Mr. A. THIRUGNANA SAMBANTHAN, M.Pharm., (Ph.D.)** Assistant Professor, Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, during the academic year 2011-2012.

Place: Melmaruvathur
Date:

Prof. (Dr.). T. VETRICHELVAN, M.Pharm., Ph.D.,
Principal,
Adhiparasakthi College of Pharmacy,
Melmaruvathur- 603319.

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Words are not just enough to express our gratitude to the blessing of **GOD** throughout the work. Indeed our project is a small work done with the help of primitive persons at heart. So it is my bounded duty to promulgate them individually.

With great respect and honor, I extend my thanks to **Thirumathi LAKSHMI BANGARU ADIGALAR,** Vice President, ACMEC Trust, Melmaruvathur for this opportunity and support all the way in completing the study.

I got inward bound and brainwave to endure experimental investigations in synthetic chemistry to this extent; I concede my most special gratitude and thanks to **Mr. A. THIRUGNANA SAMBANTHAN, M.Pharm., (Ph.D.)** Assistant Professor**,** Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy for the active guidance, valuable suggestions and a source of inspiration for my work.

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I am indeed very much thankful to the librarian **Mr. M. Suresh, M.L.I.S.,** for providing all reference books for completion of this project.

I am thankful to all my colleagues, juniors, seniors and my friends for their support and suggestion during my work.

Finally yet importantly, I one word forward my affectionate thanks to my family members, especially my parents, brother and sisters for their frequent prayers which has sustained me a lot in the successful completion of my project work**.**

SANKARNARAYANAN. S

DEDICATED TO MY PARENTS AND GOD

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ABBREVIATIONS

Ala	-	Alanine
Gly	-	Glycine
Phe	-	Phenyl alanine
Leu	-	Leucine
BOC	-	Tert-butyloxy carbonic anhydride
IPA	-	Iso propyl alcohol
DCM	-	Dichloro methane
THF	-	Tetrahydro furan
TEA	-	Triethyl amine
EDC	-	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
RT	-	Room temperature
M.P	-	Melting point
R_f	-	Retardation factor
m.mol	-	Millimole
g	-	Gram
ml	-	Milli litre
ppm	-	Parts per million
IR	-	Infra Red spectroscopy
^{1}HNMR	-	Proton Nuclear Magnetic Resonance spectroscopy
^{0}C	-	Degree Celsius
h	-	Hour
min	-	Minutes

INTRODUCTION

I. INTRODUCTION

The name quinazoline (German: Chinazolin) was first proposed for this compound by Weddige, on observing that this was isomeric with the compounds cinnoline and quinoxaline. Paal and Bush suggested the numbering of quinazoline ring system, which is currently used. The other less commonly used names for this ring system are 'phenmiazine' and 5,6-benzopyrimidine.

Of many derivatives of quinazoline system known so far, keto-quinazolines also called as quinazolinones, are the most important compounds. Depending upon the position of the keto or oxo group, these compounds may be classified into two types: 2-(1H) quinazolinones (or) 1, 2-dihydro-2-oxo quinazolines and 4(3H)-quinazolines or 3, 4-dihydro-oxoquinazolines. These systems exhibit lactam-lactim tautomerism and undergo hydroxyl group replacement reactions. 2-Cyano-4(3H)-quinazolinone was the first quinazolinone derivative to be synthesized.

Reactivity of 4(3H)-Quinazolinones

Reactions associated with tautomeric nature of the quinazolinones are often quite complex and generally unpredictable. The recorded chemical investigation on the subject is voluminous. The amide linkages in quinazolinones should not be looked on as predominantly the keto or the enol form but as true keto-enol tautomers, showing reaction characteristic of both the forms.

Quinazolinones are always high melting crystalline solids, insoluble in water and in most organic solvents but soluble in aqueous alkali. They are generally insoluble in dilute acids but are sometimes soluble in concentrated acids. Simple 4(3H)-quinazolinones, although insoluble in dilute acids, are soluble in 6N hydrochloric acid. 4(3H)-quinazolinones form stable monohydrochlorides,

chloroplatinate, chloroaurates and picrates and their metal salts of silver, mercury, zinc, copper, sodium and potassium.

Stability of the ring system

The ring system in quinazolinone is exceedingly stable in oxidation, reduction, hydrolysis reactions and other treatment designed to break the ring. There is no report of degradation of quinazolinone by simple chemical oxidation.

Biological importance of 4(3H)-Quinazolinones

The quinazolinone skeleton is a frequently encountered heterocycle in medicinal chemistry literature with applications including antibacterial, analgesic, antiinflammatory, antifungal, antimalarial, antihypertensive, CNS depressant, anticonvulsant, antihistaminic, local anaesthetic, antiparkinsonism, antiviral and anticancer activities. Little number of quinazolinones was reported as potent chemotherapeutic agents in the treatment of tuberculosis. For example 3-aryl-6, 8-dichloro-2*H*-1,3-benzoxazin-2,4(3*H*)-diones and 3-arylquinazolin-2,4(1*H*,3*H*)-diones as antimycobacterial agents, quinazolinone derivatives as antitubercular agents. *(Dr. S. N. Meyyanathan, Pharmainfo.net., 2005).*

Research programs for the discovery of new antimicrobial drug for improving the evaluation criteria are under way in many laboratories. In addition, knowledge of specific constituents of the mycobacterial cell and their biochemical role has advances considerably in the recent years and may permit a more rational approach to the design of new drug acting on specific targets. Also recent improvements in the knowledge of the mechanism of action of the available drug in the biochemical mechanism of resistance to them may be used as a basis for designing new and better weapons to fight the mycobacterial diseases.

The quinazolines and quinazolones when selectively functionalized act as building blocks for the preparation of numerous biological active compounds. The quinazolines are found to exhibit antifungal, antitubercular, antitumour and anticonvulsant activities. *(Shahikant R Pattan., et al., 2006).*

The need of new antimicrobial agents is justified because more microorganisms are being resistance to the present drugs available in the market. Worldwide researchers are trying to synthesize new drugs with better pharmacokinetic and dynamic properties with less adverse effects. The literature survey suggests that the 2, 3-substituted quinazolin-4-ones have proved to be the good bioactive molecules. They have shown diverse biological activities like antibacterial, antifungal, antiinflammatory, antitubercular, anticonvulsant, antiHIV, cardiac stimulant, diuretic and anticancer activity etc. *(Jayshari S Pattan., et al., 2009).*

Pharmacologically, quinazolin-4-ones are most important classes of heterocyclic compounds. These compounds possess versatile type of biological activities. Structure activity relationship studies of quinazolinone ring system revealed in various literatures suggest that position 2, 6 and 8 are very much important for structure activity studies. *(Raghavendra N.M., et al., 2008).*

The exploitation of a simple molecule with different functionalities for the synthesis of heterocyclic is a worthwhile contribution in the chemistry of heterocycles. Quinazolones have been frequently used in medicine because of their wide spectrum of biological activities. In light of the growing number of applications in recent years, there has been an enormous increase in the interest among biologists and chemists in their synthesis and bioactivity of quinazoline derivatives.

Analgesics are the drug which relieves pain without disturbing consciousness. Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances either in the body or in the external environment of an individual and thus has protective function. (*Gupta S. K ., 2004*)

Analgesics are classified into opioid and Non opioid analgesics. The term opioid is used to denote all naturally occurring, semisynthetic and synthetic drugs, which have morphine like action. These drugs are called Narcotic analgesics. e.g. morphine and their derivatives, codeine and its derivatives, synthetic compounds such as Pethidine, Methadone, Propoxyphene, Levorphanol and Tramadol.

Opioid drugs produce their effects by binding to opioid receptors, which are widely distributed in CNS and other tissues. Opioid receptors are also present in the peripheral nerves where they probably respond to peripherally applied opioids and locally released endogenous peptides during inflammation.

Non opioid analgesics are the agents which do not interact with opioid receptors and relieve pain without depressing the CNS. e.g. Salicylates and related compounds. Efforts towards the development and identification of new molecules for analgesic and antiinflammatory activities with minimal gastrointestinal ulceration side effects have gained lot of significance. There are no promising quinazolines which are in the market in these NSAIDS category except few drugs like Proquazone, Afloqualone and Diproqualone etc.

The novel derivatives of quinazolines mentioned might be beneficial in terms of biological activity for which further studies can be done to confirm it as a potential drug candidate. Proquazone a derivative of quinazolin-4-one exhibits potential NSAID which has been used in the disease conditions like rheumatoid arthritis,

ankylosing spondylitis, osteoarthritis, inflammatory conditions and acute pain states such as dysmenorrhea, postoperative pain and headache. Its derivative fluproquazonal has potent analgesic action. *(Vijaychand et al., 2011)*

Bacteria are microorganisms that have circular double-stranded DNA and (except for mycoplasmas) cell walls. Most bacteria live extracellularly. Some bacteria eg. *Salmonella typhi*, *Neisseria gonorrhoeae*, *Legionella*, *Mycobacterium*, *Chlamydia*, and *Chlamydophila* spp preferentially residue and replicate intracellularly. Some bacteria such as chlamydiae and rickettsiae are obligate intracellular pathogens (i.e., able to grow, reproduce, and cause disease only within the cells of the host). Others (eg, *Salmonella typhi*, *Brucella* sp, *Francisella tularensis*, *N. gonorrhoeae*, *N. meningitidis*, *Legionella* and *Listeria* spp, *Mycobacterium tuberculosis*) are facultative intracellular pathogens.

Antibacterial drugs are derived from bacteria or molds are synthesized by de novo. Technically, antibiotic refers only to antimicrobials derived from bacteria or molds but is often including used synonymously with antibacterial drug.

Antibiotics have many mechanisms of action, including inhibiting cell wall synthesis, activating enzymes that destroy the cell wall, increasing cell membrane permeability and interfering with protein synthesis and nucleic acid metabolism.

Antibiotics sometimes interact with other drugs. Raising or lowering serum levels of other drugs by increasing or decreasing their metabolism or by various other mechanisms.

The rapid rise in bacterial resistance to the traditional antibiotics such as penicillins and tertracyclines has encouraged a continuing search for new classes of

compounds with novel modes of antibacterial activity.

In view of the above reports, the design and synthesis of newer antimicrobials is an area of immense significance and continues to attract the attention of increasing number of medicinal chemists.

With the interest of on quinazolin-4-ones due to its extension pharmacological actions we have designed a new series of quinazolinone substituted at 2^{nd} position with chloromethyl and 3^{rd} position with different amino acid and heterocycles.

OBJECTIVE OF THE WORK

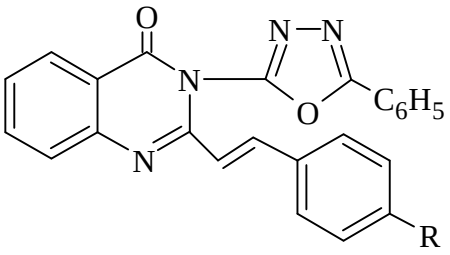
II. OBJECTIVES OF THE WORK

- To synthesize 2-chloromethyl-3-amino acid substituted quinazolin-4(3*H*)-one.
- To synthesize different 2-chloromethyl-3-heterocyclic substituted Quinazolones by cyclizing the chloroacetyl anthranilic acid with amino substituted indole, benzthiazole and pyridine.
- To characterize the structures of the synthesized compounds by various spectroscopic methods via IR, NMR and Mass spectroscopy.
- To screen the analgesic activity of synthesized compounds by Eddy's hot plate method.
- To evaluate the antimicrobial activity of synthesized compounds by Disc Diffusion method.
- To interpret and conclude the results obtained from the study.

LITERATURE REVIEW

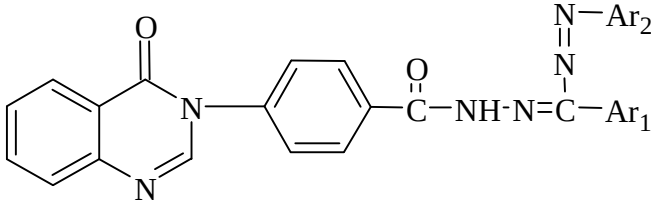
III. LITERATURE REVIEW

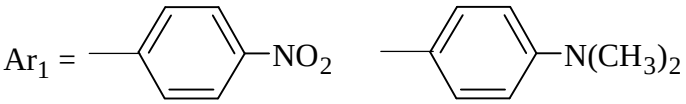
Rajitha G. *et al.*, (2011) synthesized some novel 3-[5-phenyl-1,3,4-oxadiazol- 2-yl]-2-(substituted styryl)-quinazolin-4(3H)-ones and evaluated for antibacterial activity.

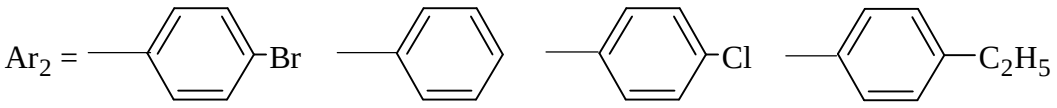


R = H, OCH_3, CH (CH) $_{3,}$ Cl, N $(CH_3)_{2,}$ $NO_{2,}$ CH_3

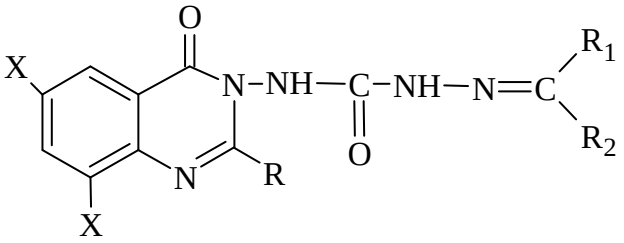
Narendra babu *et al.*, (2011) carried out the synthesis of new quinazolinone and the compounds were screened for antiinflammatory and anthelmintic activities.



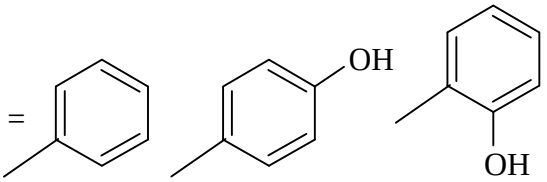




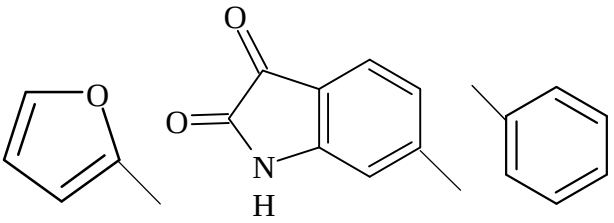
Ponnilavarasan Ilangovan *et al.*, (2010) synthesized a series of different quinazolinone derivatives, and they were examined by invitro studies for anticonvulsant and antimicrobial activities.



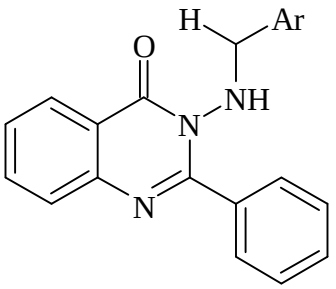
R =



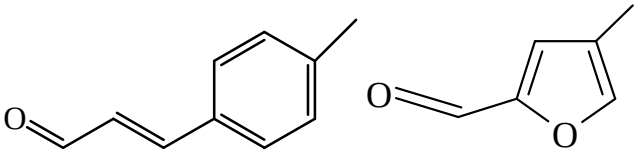
R_1 , R_2 =



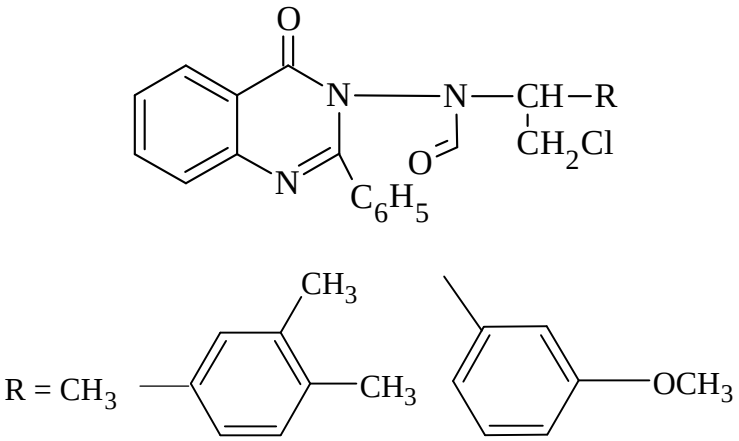
Mariappan G. *et al.*, (2010*)* synthesized a series of some unique quinazolone fused schiff bases and those compounds were evaluated for biological activity.



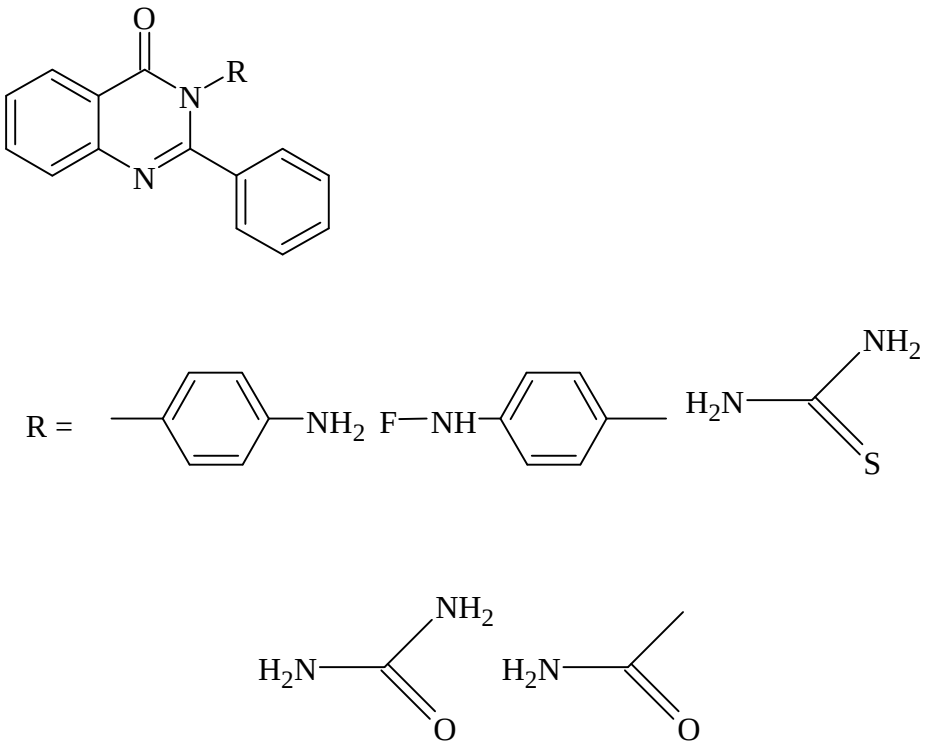
Ar =



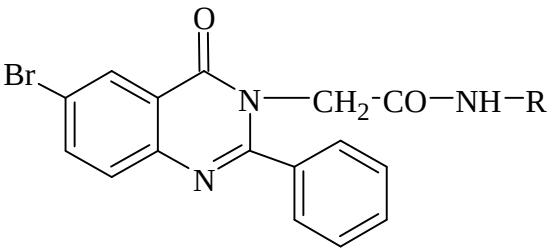
Beena K. P. *et al.*, (2010) synthesized new series of quinazolinone derivatives for antibacterial activity.



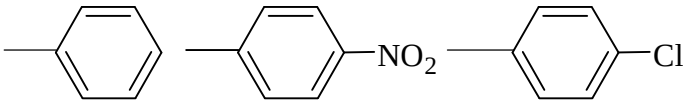
Kale A. U. *et al.*, (2010) synthesized some new 2, 3-disubstituted quinazolin-4-(3H)-ones and they were tested for *invitro* antimicrobial activity.

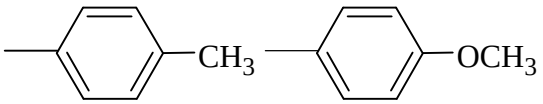


Rajveer C. H. *et al.*, (2010) carried out the synthesis of 2-(6-bromo-2-phenyl-4-oxoquinazolin-3(4H)-yl)-N-substituted acetamides and 1-amino-5-(6-bromo-3, 4-dihydro-2-phenyl-4-oxoquinazolin-3yl) methly-1,3,4-triazin-2-thiol and the selected compounds were examined for antimicrobial activity.

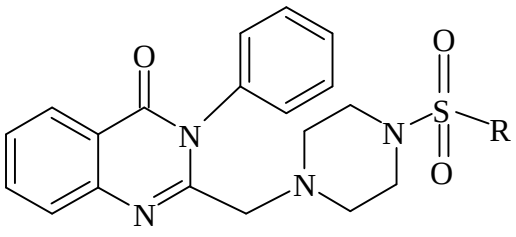


R =



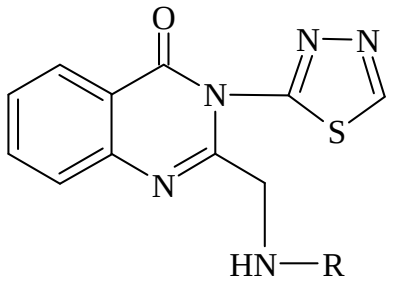


Dubey *et al.*, (2010) synthesized a novel series of 2-(4-substituted sulfonyl piperazin-1-ylmethyl-3-aryl-quinazolin-4(3H)-one and screened for the antimicrobial activity.



R = CH_3, C_2H_5, C_3H_7

Shashikant R. Pattan *et al.*, (2009) synthesized some new quinazolone derivatives and the proposed compounds were screened for antimicrobial, antifungal, antitubercular and antiinflammatory activities.



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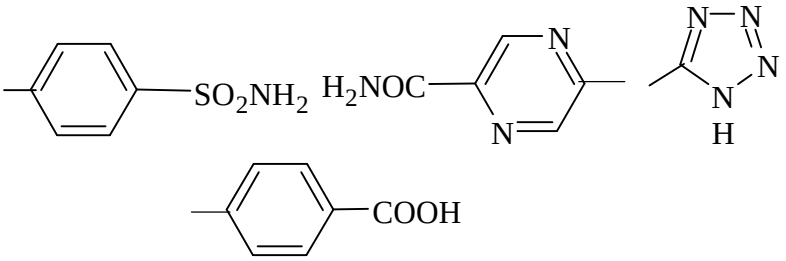
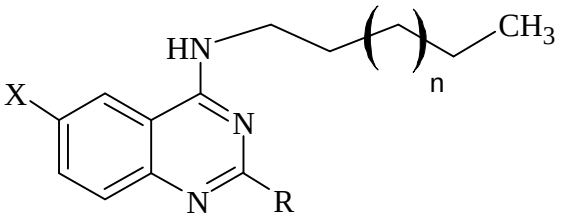


Narsaiah B. *et al.*, (2009) carried out the synthesis of novel 2, 4, 6 trisubstituted quinazoline derivatives showing antibacterial and cytotoxic activities against THP-1, HL-60 and A375 cell line.

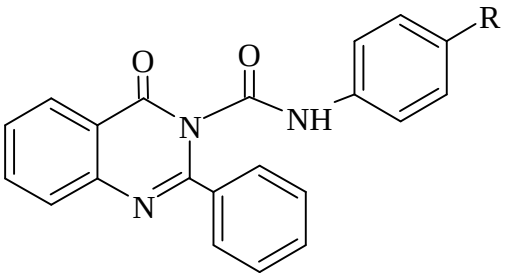


X = H, Br, I

R = CH_3, CF_3, C_6H_5, $C_6H_4F_3$

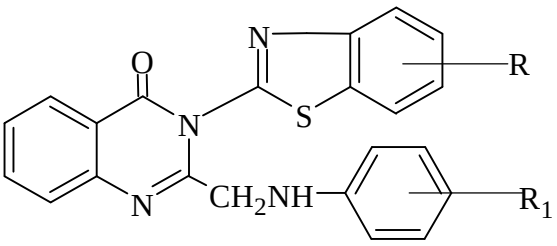
Vijayanand R. *et al.*, (2009) synthesized a series of 4-oxo-2-phenyl-4H quinazolin-3-carboxylic acid (4-substituted phenyl amides) by condensing 2-phenyl-3, 1-

benzoxazin-4one and 4-substiuted phenyl urea. The compounds were found to have significant effect against bacterial and fungal organisms.



R = Cl, Br, NO_2, SO_2NH_2, COOH

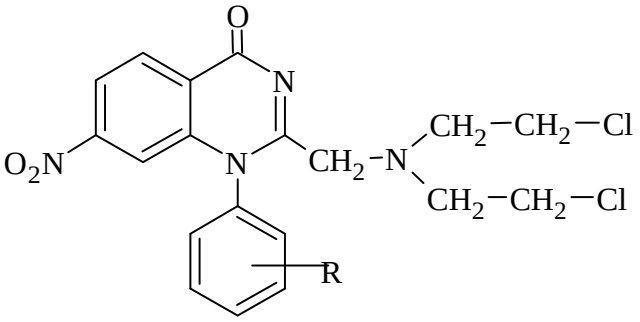
Salahuddin M. D. *et al.*, (2009) synthesized some novel 3-(6-Substituted-1, 3-benzothiazol-2-yl)-2-[{(4-substituted phenyl) amino} methyl} quinazolin-4(3H)-ones with various substituted amine and selected derivatives are investigated for antiinflammatory and antibacterial activities.



R = CH_3, OCH_3, Cl, F

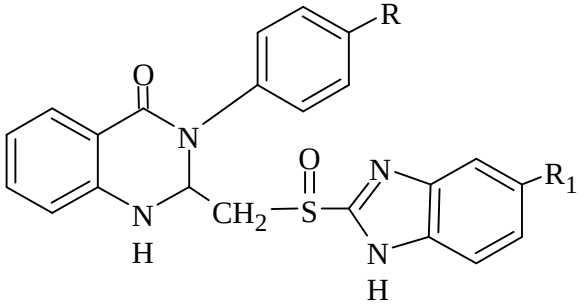
R_1 = H, CH_3, OCH_3, Cl,

Gowramma B. *et al.*, (2009) synthesized few compounds of 1-sustituted phenyl 2-[(bis-(2-chloroethyl)amino]-methyl-7-nitro-4(1H)-quinazolinone by chlorination with phosphorous oxychloride and phosphorous penta chloride and all the compounds were incorporated with nitrogen mustard moiety and screened for anticancer activity.



R = OCH_3, OC_2H_5, ONO_2, -H, -CH_3, -$N(CH_3)_2$.

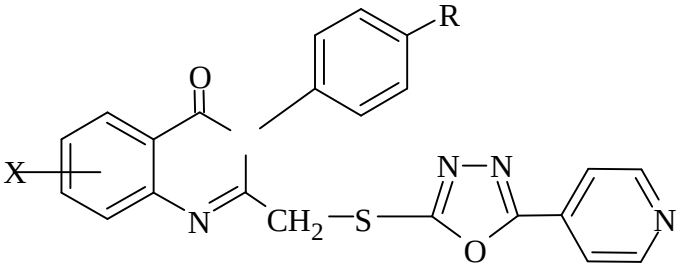
Avinsah patil *et al.*, (2009) carried out a new synthesis of 2-[{5-substituted-1H-benzimidazol-2-yl sulfinyl} methyl]-3-substituted Phenyl Quinazolin-4(3H)-one derivatives; antiulcer activity of the derivatives were studied by pylorous ligation induced ulcer models in rats.



R = H, CH_3, OC_2H_5, Br, Cl

R_1= H, OCH_3, OCH_2F

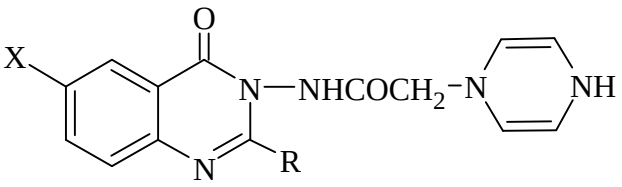
Jai singh *et al.*, (2008) synthesized an innovative series of 2-{5′-(4-Pyridinyl)-1′,2′,3′-oxadiazol-2-yl-thiomethyl]-3-substituted-aryl-6-substituted-quinazolin-4-one and all the compounds were screened for antiinflammatory activity.



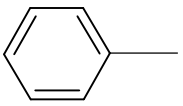
X = H, Br, I

R = H, Cl, OCH_3,

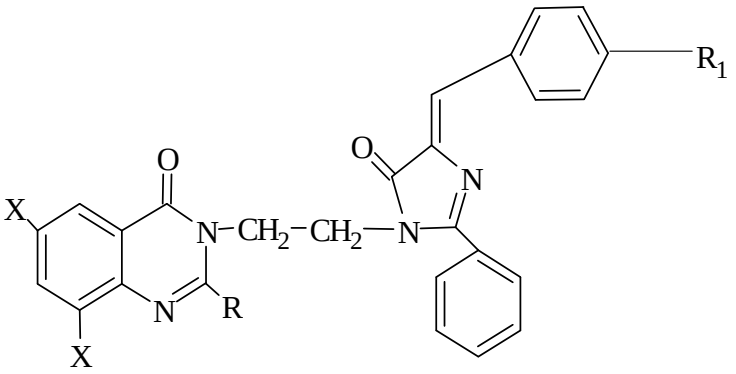
Raghavendra M. N. *et al.*, (2008) synthesized by condensation of 2-chloro-*N*-(4-oxo-substituted-quinazolin-3(4*H*)-yl)-acetamides with various substituted piperazines through single step reaction and the derivatives were investigated for antibacterial and antifungal activities.



X = H, Br

R = CH_2, CH_2-CH_2-CH_3, 

Suthakaran R. *et al.*, (2008) synthesized a different sequence of 3-(2-(4)-4-substituted benzylidene-4, 5-dihydro-5-oxo-2-phenyl imidazol-1-yl) ethyl)-6, 8-un/dibromo subtituted-2-substituted quinazolin-(3H)-one and compounds were investigated for antibacterial and antifungal activities

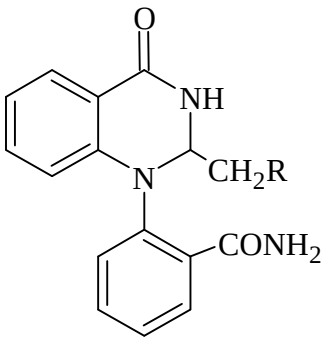


X = H, Br

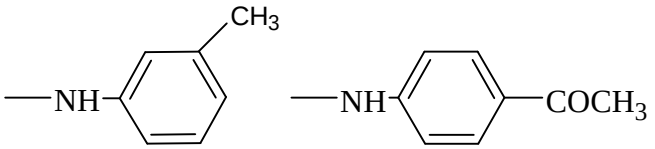
R = CH_3, CH_2Cl, C_6H_5

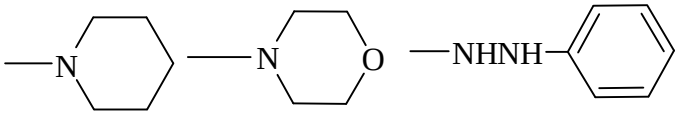
R_1 = OH, Cl

Srinivasa reddy *et al.*, (2007) synthesized a series of 1-phenyl-2-(substituted)-4-(1H) quinazolinones and the compounds were screened for in vivo analgesic and antiinflammatory activities.

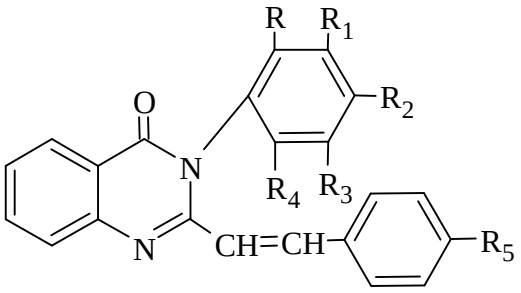


R =





Jessy E. M. *et al.*, (2007) synthesized a unique series of 2, 3-disubstituted-3, 1-quinazolin-4-(3H) ones and the compounds were screened for antibacterial and antiinflammatory activities.



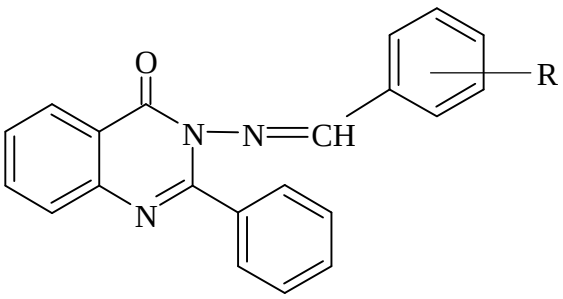
$R = R_1 = H, COOH$

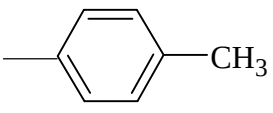
$R_2 = H, Br$

$R_3 = R_4 = H, Cl$

$R_5 = NO_2, OCH_3, OH$

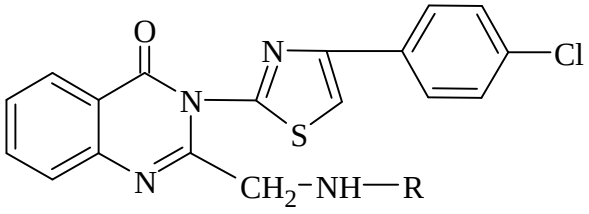
Ashis Kumar Nanda *et al.*, (2007) synthesized of ten 3-(arylidene amino)-2-phenylquinazolin-4(3H)-ones with QSAR studies are carried out and the compounds were investigated for antimicrobial activity.

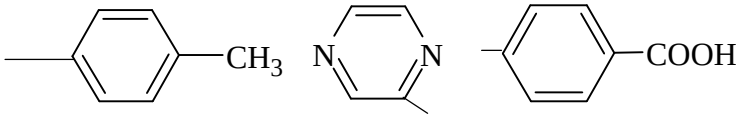




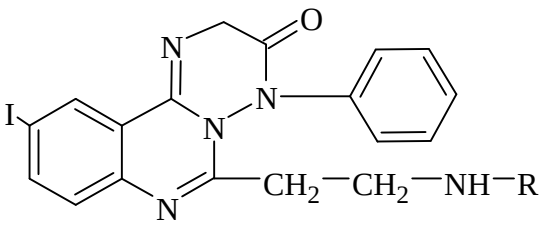
$R = CH_3, OH, F, Cl, NO_2.$

Shashikant R. Pattan *et al.*, (2006) synthesized a new series of N-3[(4-(4-chlorophenyl thiazole-2-yl)-(2-(amino) methyl)-quinazolin-4(3H)-ones and all the derivatives were screened for their antitubercular activity.



R = 

Tripti singh *et al.*, (2006) synthesized a series of heterocyclic derivatives of quinazolinone and the compounds were screened for insecticidal and antimicrobial activities.



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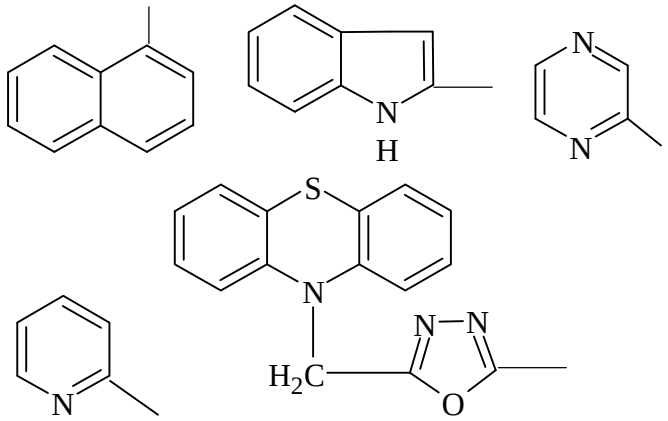
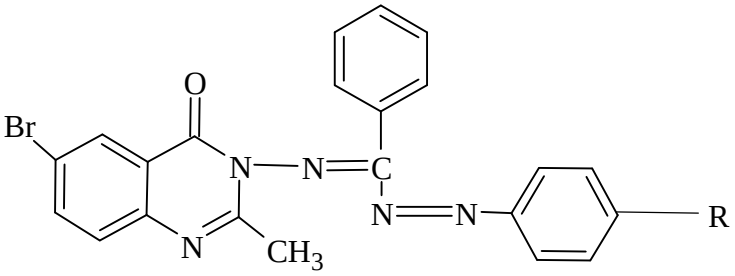
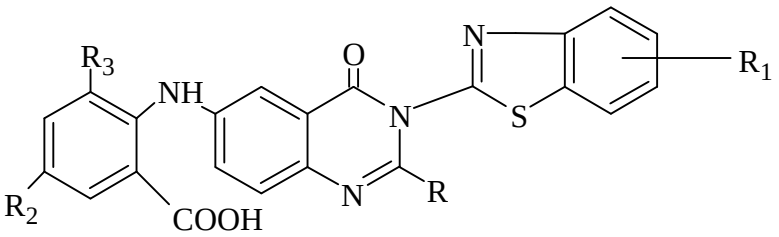


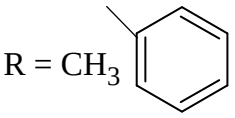
Mistry D. *et al.*, (2006) synthesized of newer quinazolinones and the compounds were screened for antimicrobial activity.



R = H, OCH_3, Br, Cl, NO_2,

Manish Patel *et al.*, (2005) carried out the synthesis of new 2, 3, 6-trisubstituted quinazolin-4(3H)-ones and antibacterial potential was evaluated for the selective moieties.

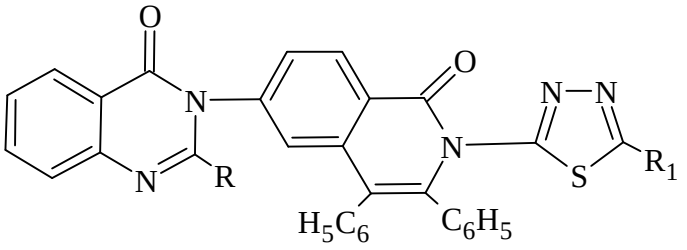


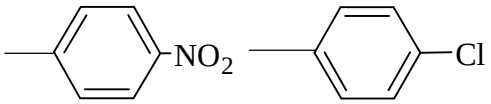


$R_1 = OCH_3$

$R_2 = R_3 = H, Br$

Pandey D. *et al.*, (2005) synthesized a new series of isoquinolinyl quinazolones and investigated all the compounds for antiviral and antifungal activities.

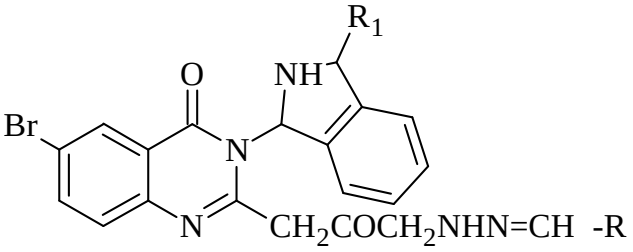




$R = CH_3, C_2H_5, C_5H_5$-CH=CH,

$R_1 = CH_3, C_2H_5$

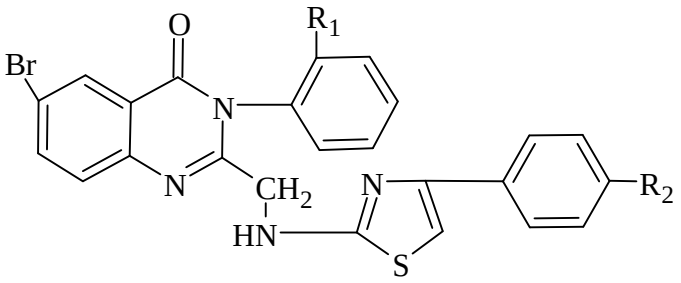
Ashok kumar *et al.*, (2003) synthesized some new 2, 3, 6 trisubstituted quinazolinone for potent antiinflammatory, analgesic and COX-2 inhibitory activities.



R= H, CH_3, OCH_3

$R_1 = CH_3$, C_2H_5, C_6H_5, Br, Cl

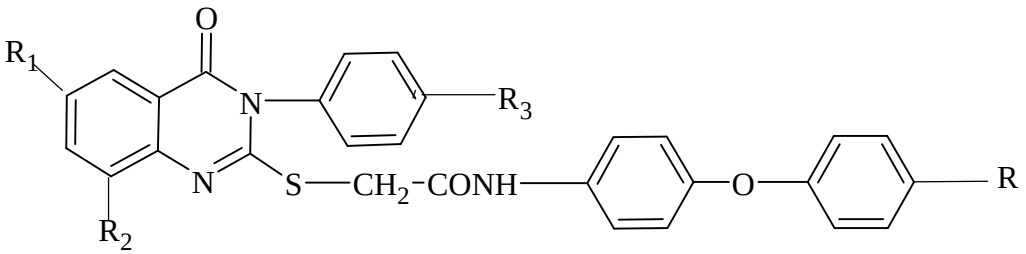
Bhatta V. *et al.*, (1998) synthesized new sequences of 3-aryl-2-(4′-aryl thiazol-2′-yl aminomethyl quinazolin-4(3H)-ones and all the compounds were investigated for antimicrobial activity.



R_1 = H, CH_3, OCH_3, Cl

R_2 = CH_3, OCH_3, Cl

Surendara bahadur *et al.*, (1982) synthesized of some newer-4(3H)-quinazolinones and the compounds were examined for antimicrobial agents.



R = H, CH_3

R_1 = R_2 = H, Br

R_3 = H, CH_3, OCH_3, Cl

IV. EXPERIMENTAL SECTION

A. MATERIALS AND METHODS

List of chemicals and instruments used for the study:

Table-1

S. No	Chemicals / Instruments	Supplier / Model
	Chemicals	
1.	Alanine	Spectrochem Pvt. Ltd.,
2.	Glycine	Lobachem Pvt. Ltd.,
3.	Leucine	Spectrochem Pvt. Ltd.,
4.	Phenyl alanine	Lobachem Pvt. Ltd.,
5.	Anthranilic acid	Sigma Aldrich
6.	Chloroacetyl chloride	Lobachem Pvt. Ltd.,
7.	Benzene	Lobachem Pvt. Ltd.,
8.	Hydrazine hydrate	Lobachem Pvt. Ltd.,
9.	Ethanol	Lobachem Pvt. Ltd.,
10.	Acetone	Lobachem Pvt. Ltd.,
11.	Pyridine	Lobachem Pvt. Ltd.,
12.	Benzimidazole	Lobachem Pvt. Ltd.,
13.	Glacial acetic acid	Spectrochem Pvt. Ltd.,
14.	t-butyl oxy carbonic anhydride	Qualigens Fine Chemicals
15.	Iso propyl alcohol	Lobachem Pvt. Ltd.,
16.	Chloroform	Spectrochem Pvt. Ltd.,
17.	Petroleum ether (40-60^{0}c)	Qualigens Fine Chemicals

18.	Thionyl chloride	Spectrochem Pvt. Ltd.,
19.	Sodium hydroxide	Spectrochem Pvt. Ltd.,
20.	Methanol	Qualigens fine Chemicals
21.	Dichloro methane	Lobachem Pvt. Ltd.,
22.	Tetrahydro furan	Lobachem Pvt. Ltd.,
23.	Trifluroacetic acid	Qualigens Fine Chemicals
24.	Sodium sulphate anhydrous	Qualigens Fine Chemicals
25.	Conc. sulphuric acid	Lobachem Pvt. Ltd.,
26.	Conc. hydrochloric acid	Qualigens Chemicals
27.	Diethyl ether	Qualigens Fine Chemicals
	Instruments	
28.	Magnetic stirrer	Remi Equipments
29.	Weighing balance	Shzimadzhu 220 v
30.	Melting point apparatus	Sun bim Equipments
31.	Hot air oven	Picses Instruments
32.	Heating mantle	Ajay Equipments

Methods

- Melting points were determined by using open capillary tubes and were uncorrected.
- The completion of reaction and purity of the compounds were checked by thin layer chromatography on pre-coated silica gel plates using iodine vapours as detecting agent.

- The I.R spectra of the synthesized compounds were recorded in JASCO FT-IR spectrophotometer in Ideal Analytical Lab, Puducherry.

- The NMR spectra of the synthesized compounds were recorded in BRUKER 400 MHz NMR spectrometer, SAIF, Punjab University, Punjab.

- The Mass spectra of the synthesized compounds are recorded in JEOL GCmate using Electron impact method as ionization mode in SAIF, IIT-Madras.

- The analgesic activity of the synthesized compounds was screened by Eddy's Hot plate method in Adhiparasakthi College of Pharmacy, Melmaruvathur.

- The antimicrobial activity of the synthesized compounds was screened by Pharma Analytical Lab, Puducherry.

Figure-1 **B. SCHEME OF THE WORK**

SCHEME I

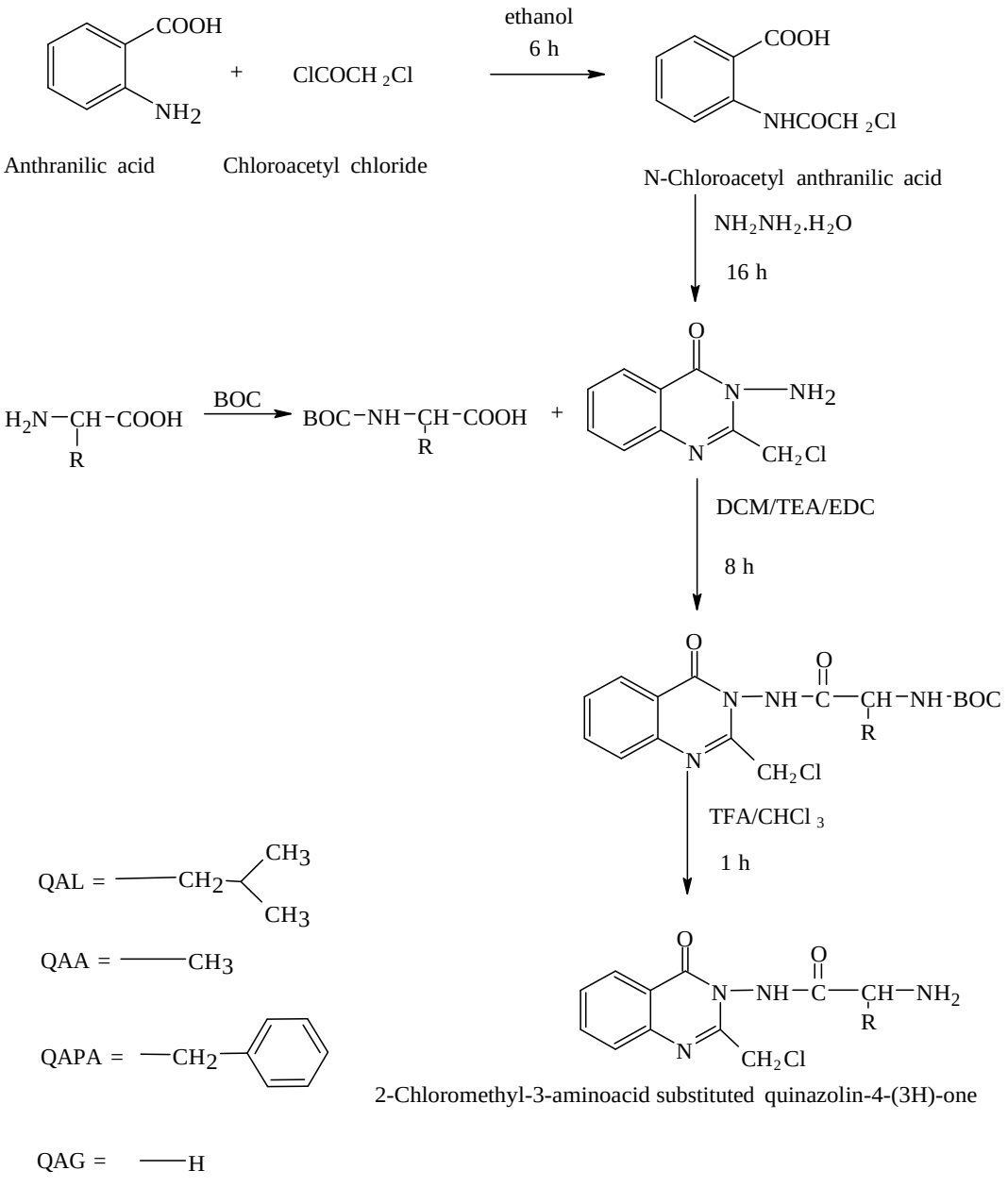
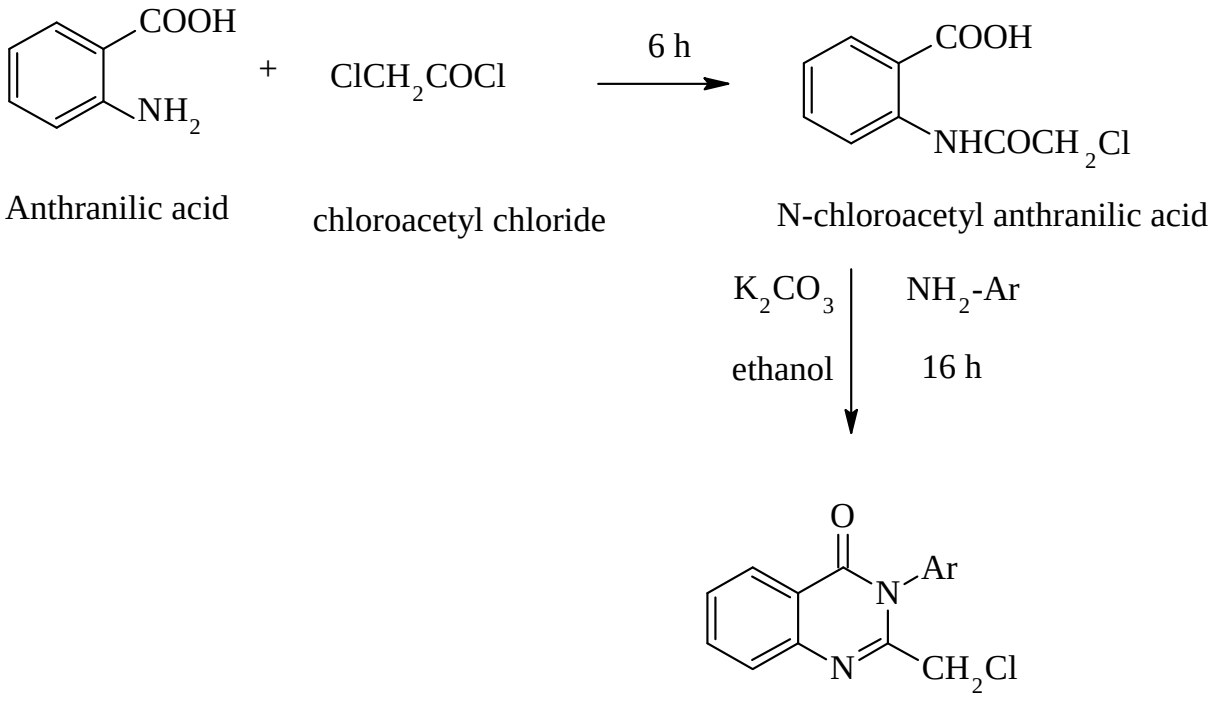


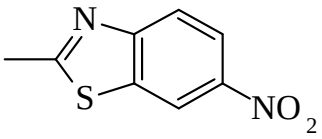
Figure-2

SCHEME II

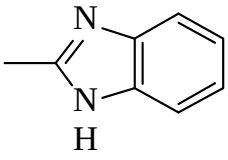


Ar =

QBEN =



QIBM =



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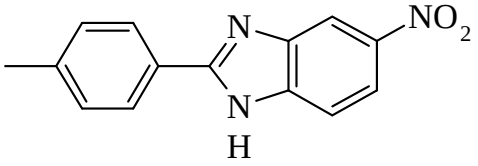
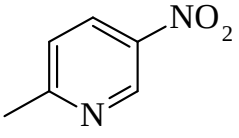
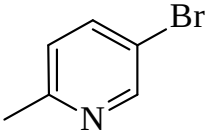


QPIN =



QBrP =

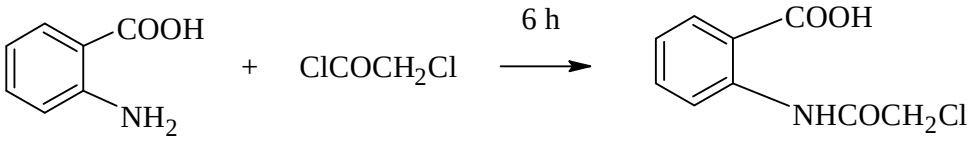


C. METHADOLOGY

Procedure for scheme I

Synthesis of N-chloroacetyl anthranilic acid:

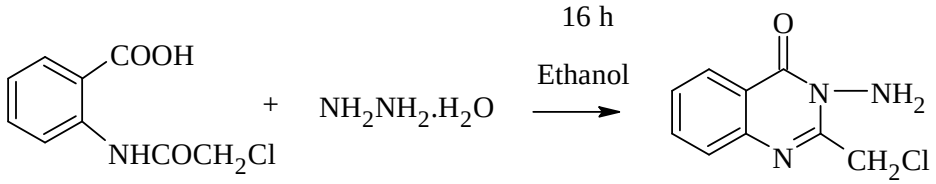
0.1M (13.7gm) of anthranillic acid was taken in a 250 ml round bottom flask followed by addition of 0.15M (16.95ml) chloroacetyl chloride in 80ml of benzene and two to three drops of pyridine under cold condition. The reaction mixture is refluxed for 6 h cooled and filtered it. The solid was purified by recrystallization from acetone-ethanol mixture (1:1). *(Shashikant R Pattan., et al., 2006*)



Anthranillic acid Chloroacetyl chloride N-Chloroacetyl anthranilic acid

Synthesis of 3-amino-2-(chloromethyl) quinazolin-4(3*H*)-one):

N-Chloroacetyl anthranilic acid (0.01M) was refluxed with Hydrazine hydrate (0.01M) in the presence of 10g of K_2CO_3 in 100ml of dry ethanol for 16 h. The ethanol was evaporated and the mixture was neutralized with 0.1M HCl under ice cold condition. Then the residue was washed thoroughly with boiling water and recrystallized from acetone: ethanol mixture (1:1). *(Shashikant R Pattan., et al., 2006*)



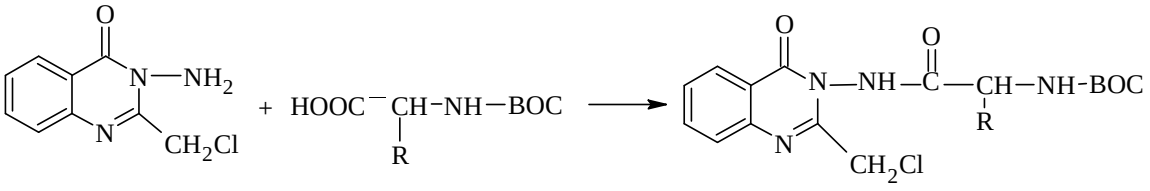
N-Chloroacetyl anthranilic acid Hydrazine hydrate 3-amino-2-(chloromethyl) quinazolin-4(3H)-one)

Protection of Amino Group

The amino acid (20 m.mol) was dissolved in a mixture of 1N NaOH (20 ml) and IPA (20 ml). To this BOC (26 m.mol, 6 ml) in IPA (10 ml) was added, followed by 1N NaOH (20 ml) to the resulting solution. The solution was stirred at room temperature for 2 h, washed with light petroleum ether (40 – 60^0C) (20 ml), acidified to pH-3 with 2N sulphuric acid and finally extracted with chloroform (3 x 20 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated under pressure to give the BOC-amino acid. The crude product was recrystallized using a mixture of chloroform and petroleum ether. (*Bodanszky. M., 1984*)

Coupling of quinazolone with BOC-amino acid

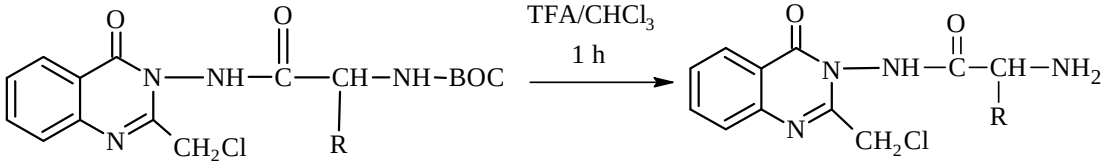
3-amino-2-(chloromethyl) quinazolin-4(3*H*)-one) (10 m.mol) was dissolved in DCM (20 ml). To this triethyl amine (4 ml) was added at 0°C and the reaction mixture was stirred for 15 minutes. BOC- amino acid (10 m.mol) in chloroform (20 ml) and EDC (10 m.mol) were added with stirring. After 8 h the reaction mixture was filtered and residue was washed with chloroform (30 ml) and added to the filtrate. The filtrate was washed with 5% sodium bicarbonate (20 ml) and saturated sodium chloride (20 ml) and water. Organic layer was dried over anhydrous sodium sulphate and the product was recrystallized by chloroform and petroleum ether. (*Bodanszky. M., 1984*)



3-amino-2-(chloromethyl) quinazolin-4(3H)-one

Synthesis of 3-amino acid substituted-2-chloromethyl quinazolin-4(3*H*)-one

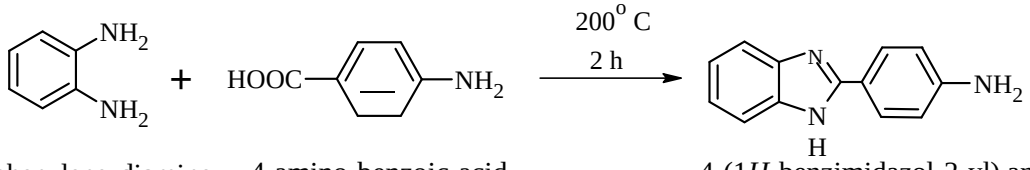
The protected amino acid was dissolved in chloroform (15 ml) and treated with trifluro acetic acid (2 m.mol). The solution was stirred at room temperature for 1h and washed with saturated sodium bicarbonate (5 ml) and the organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. (*Bodanszky. M., 1984*)



Procedure for scheme II

Synthesis of (1*H*-benzimidazol-2-yl) aniline:

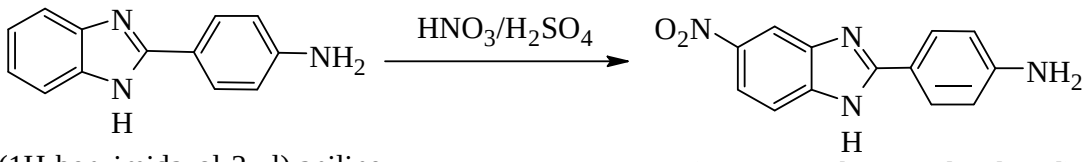
A mixture of O-phenylene diamine 3.8 g (34 mM) and 4-amino benzoic acid 4.5 g (33 mM) were stirred in a syrupy ortho-phosphoric acid (45 ml) at 200º C for 2 h. The reaction mixture was cooled and poured on to the crushed ice. The bulky white precipitate obtained was stirred in cold water (400ml) and sodium hydroxide solution (5 M) was added until the pH 7. The resulting solid was filtered and recrystallized from methanol. (*Sreena et al., 2009*)



O-phenylene diamine 4-amino benzoic acid 4-(1*H*-benzimidazol-2-yl) aniline

Synthesis of 4-(5-nitro-1*H*-benzimidazol-2-yl) aniline:

Concentrated nitric acid (7.5 ml) was placed in 3-necked round bottom flask fitted with a mechanical stirrer. The flask was immersed in ice cold water and added slowly concentrated sulphuric acid (7.5 ml) down the condenser with slow stirring. After the addition, (1*H*-benzimidazol-2-yl) aniline 5.85 g (0.028 M) was added in a portion over a period of 1 h at such a rate that the temperature did not exceed 35º C. After continuous stirring for 12 h, the reaction mixture was poured very slowly over crushed ice with vigorous stirring. The formed product was filtered, washed with cold water and recrystallized from ethanol. (*Jitender Singh et al., 2010*)

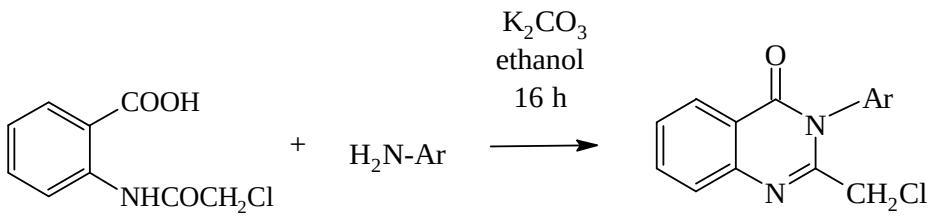


4-(1H-benzimidazol-2-yl) aniline

4-(5-nitro-1H-benzimidazol-2-yl) aniline

2-chloromethyl-3- substituted quinazolin-4(3H)-one:

N-chloroacetyl anthranilic acid (0.01M) was refluxed with (0.01M) of 2-amino benzthiazole, 2- amino benzimidazole, 2-amino 5-nitro phenyl benzimidazole, 2-amino 5-nitro pyridine and 2-amino 5-bromo pyridine in the presence of 10g of K_2CO_3 in 100ml of dry ethanol. The ethanol was evaporated and the mixture was neutralized with 0.1M HCl under ice cold condition. Then the residue was washed thoroughly with boiling water and recrystallized from acetone: ethanol mixture (1:1).



N-chloroacetyl anthranilic acid

2-chloromethyl-3-substituted quinazolin-4(3H)-one

D. EVALUATION OF ANALGESIC ACTIVITY

The drug is reflected to obligate opioid analgesic activity if it relieves pain by acting centrally to elevate pain threshold without disturbing consciousness. If it is peripherally acting analgesics, it inhibits prostaglandin synthesis and raises the threshold to pain perception. Numerous methods for evaluation of analgesic activity are available. They can be categorized into chemical, electrical, mechanical and thermal methods. (*Gupta S .K., 2004*)

Chemical Method: Writhing Test, Randall - Selitto Test and Intra Arterial Bradykinin Test.

Electrical Method: Electrical Stimulation of the Tail, Flinch Jump test in Mice and Tooth Pulp Stimulation in Rabbits

Mechanical Method: Haffner's Tail-clip test in Mice

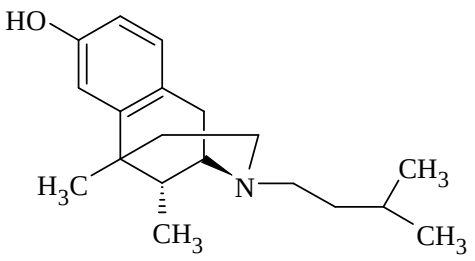
Thermal Method: Radiant Heat Method, Tail-Immersion Test and Hot plate Method.

The thermal method was followed to screen the analgesic activity. In this method the pain is induced by heat from Eddy's Hot plate.

Standard Drug for Analgesic Activity

To screen and compare the analgesic activity of synthesized compounds, the pentazocine was used as a standard drug.

Standard drug



Pentazocine

Pentazocine is the one of the potent opioid analgesic. It relieves moderate to severe pain by acting centrally to elevate pain threshold without disturbing consciousness.

Procedure for Eddy's Hot Plate Method

Albino mice of either sex (20 - 25g) were grouped as control, standard and test (I-II) containing three numbers. The animals were acclimated to laboratory conditions for one week prior to the experiment. Eddy's hot plate with the temperature of 55° ± 1°C used for the study. Mice were placed on hot plate and the reaction time that is licking of paw or jumping response was recorded in seconds. The cut off time is 15 seconds and the animals not showing any response after 15 seconds were removed from the study. The test compounds were administered intraperitonially with a dose of 50 mg/kg in 1% polyethylene glycol. Standard group was administered with pentazocine intraperitonially in a dose of 50mg/kg whereas the control group was given only with 1% v/v polyethylene glycol. The reaction time was measured at the interval of 0, 30, 60, 90 and 180 min. The animal study protocol was approved by IAEC (Reg No: 409/01/CPCSEA). The results are reported in Table No. (*Eddy N. B., et al., 1953*)

E. EVALUATION OF ANTIBACTERIAL ACTIVITY

The antibacterial activity can be evaluated by the following techniques.

A) Agar streak dilution method

B) Serial dilution method

C) Agar diffusion method

Cup plate method

Cylinder method

Paper disc method

D) Disc diffusion method

The antibacterial activity of synthesized compounds were screened in the concentration of 25, 50 and 100 μg in dimethyl formamide against gram positive organism *Staphylococcus aureus* and gram negative organism *Escherichia coli* in Muller Hinton agar medium by disc diffusion method. The antimicrobial activity was evaluated by measuring zone of inhibition in mm, the details of the procedure are given below.

Preparation of Muller Hinton Agar

Composition

Beef extract	10.0 g
Casein acid hydrolysis	17.5 g
Starch	1.5 g
Agar	20.0 g
Distilled water	1000 ml

All the ingredients were taken in 1000 ml of distilled water in a conical flask and heated in a steam bath to dissolve. The pH was adjusted to 7.0 ± 0.2 and sterilized in autoclave at 15 lb at 120° C for 15 minutes. The sterile medium was poured into petridish and allowed to solidify.

Preparation of the Disks

Paper disks of 5 mm diameter and 2 mm thickness were sterilized by autoclaving at 121° C for 15 minutes. Ciprofloxacin (10 µg/ml) was used as standard antibiotic for the comparation of antibacterial activity of the synthesized compounds.

Organism used

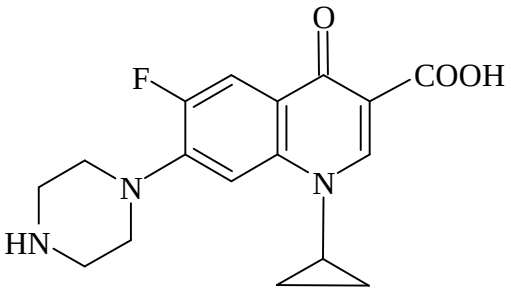
Gram positive Organisms

Staphylococcus aureus ATCC 6538P

Gram negative Organisms

Escherichia coli ATCC 2592

Standard drug



Ciprofloxacin

IV. F. EVALUATION OF ANTIFUNGAL ACTIVITY:

The antifungal activity can be evaluated by the following techniques:

Cup and plate method/ Cylinder method

Turbidimetry / Tube assay method

Organism used

Aspergillus niger ATCC 9029

The antifungal activity of synthesized compounds was screened in the concentration of 25, 50 and100 μg in dimethyl formamide against *Aspergillus niger*. The antifungal activity was evaluated by measuring zone of inhibition in mm, the details of the procedure are given below.

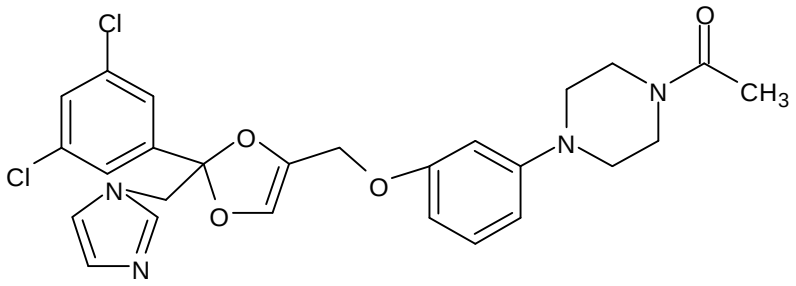
Preparation of Sabouraud's Agar Media

Composition

Dextrose	20 g
Peptone	10 g
Purified water	1000 ml
pH	5.4 ± 0.2
Agar	15 g

The media was prepared by dissolving the specified quantities of the dehydrated ingredients (Hi-media) in purified water and was distributed in petridish to a thickness of 3-4 mm. The plates were sterilized by autoclaving at 121° C for 15 minutes. The sterile medium was poured into petridish and allowed to solidify.

Standard drug



Ketaconazole

Procedure for Antimicrobial activity

The synthesized compounds were tested for antibacterial and antifungal activity by disc diffusion method at the concentration of 25, 50 and100 µg/ml. Whatmann filter paper (grade-1) disc of 5 mm diameter and 2 mm width was sterilized by autoclaving for 15 min at 121°C. The sterile agar petridish was seeded with test bacteria and the impregnated discs were placed on the medium with suitable space between the discs. The plates were incubated at 37°C ± 1°C for 18-24 h for bacterial medium and 25°C ± 1°C for 72 h for fungal medium. The inhibition of zones caused by various synthesized compounds and standard drugs ciprofloxacin and ketoconazole on the bacterial and fungal microorganisms were examined.

(*Ind. Pharmacopoeia, vol –I,* 2007).

RESULTS AND DISCUSSION

V. RESULTS AND DISCUSSION

The completion of reaction was monitored by TLC using silica gel as stationary phase and chloroform-methanol as mobile phase. The spot in the TLC plate was detected by iodine vapours. The chloroform and petroleum ether mixture was used to purify the compounds. The satisfactory yield was obtained for every reaction. The Physical and spectral data's of the synthesized compounds are given below

a. Physical data of the compounds

i) Physical Data of BOC-Amino acid

The amino group of amino acid is protected by tert-butyl oxy carbonic anhydride (BOC). The BOC amino acids were obtained with good yield. The Physical Data of the BOC-Amino acids are given below.

Table-2

BOC-amino acid	**Molecular formula**	**Molecular weight**	**Physical state**	**M. P (^oC)**	**Yield (%)**
BOC-Leu	$C_{11}H_{21}NO_4$	231.29	White powder	82-85	42.2
BOC - Ala	$C_8H_{15}NO_4$	189.21	White powder	110-113	62.0
BOC- Phe	$C_{14}H_{19}NO_4$	265.31	white powder	140-142	72.2
BOC-Gly	$C_7H_{13}NO_4$	175.80	White powder	88-90	26.2

ii) Physical Data of 2-chloromethyl-3-amino acid substituted quinazolin-4-(3H)-ones

The BOC protected amino groups are deprotected by using trifluro acetic acid in chloroform and the physical data of the compounds are given below

Table-3

Compound	Molecular formula	Molecular weight	Physcial State	Yield (%)	R_f Value
QAL	$C_{15}H_9\ ClN_4O_2$	322.78	Semisolid	52.12	0.58
QAA	$C_{12}\ H_{13}ClN_4O_2$	280.71	Semisolid	70.20	0.64
QAPA	$C_{18}\ H_{17}ClN_4O_2$	356.80	Semisolid	85.00	0.68
QAG	$C_{11}\ H_{11}ClN_4O_2$	266.68	Semisolid	70.80	0.55

Solvent system for TLC:- Chloroform : Methanol (7:3)

iii) Physical Data of 2-chloromethyl-3-heterocyclic substituted quinazolin-4(3H)-ones

Table-4

Compound	Molecular formula	Molecular weight	Physcial state	Yield (%)	M.P (°C)	R_f Value
QBEN	$C_{16}H\ ClN_4SO_3$	371.50	Brown colour	70.16	286	0.60
QIBM	$C_{16}\ H_{11}ClN_4O$	310.00	Brown colour	60.20	194	0.50
QNIBM	$C_{22}\ H_{15}ClN_5O_3$	432.83	Brown colour	65.00	274	0.80
QPIN	$C_{14}\ H_9ClN_4O_3$	316.70	Brown colour	52.00	254	0.70
QBrP	$C_{14}\ H_9BrClN_3O$	350.59	Brown colour	46.00	164	0.64

Solvent system for TLC: - Chloroform: Methanol (7:3)

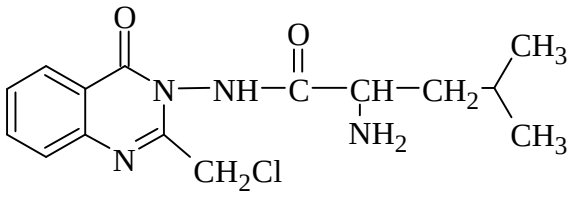
b. Spectral analysis

The synthesized compounds were characterized by IR, proton NMR and Mass spectrophotometer and the structures of the compounds are consistent with the spectra.

IR Spectral Analysis

The IR spectrum was recorded in JASCO FT-IR spectrophotometer. The significant IR values are measured in cm^{-1} and the results given in the table.

Interpretation of IR spectra of QAL



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-5

S. No	Wave numbers (cm^{-1})	Functional groups
1	3449	NH Stretching (NH_2)
2	2959	Aromatic –CH- Stretching
3	1649	C=O Stretching in amide
4	1515	C=N Stretching
5	1368	C-N Stretching
6	771	C-Cl Stretching

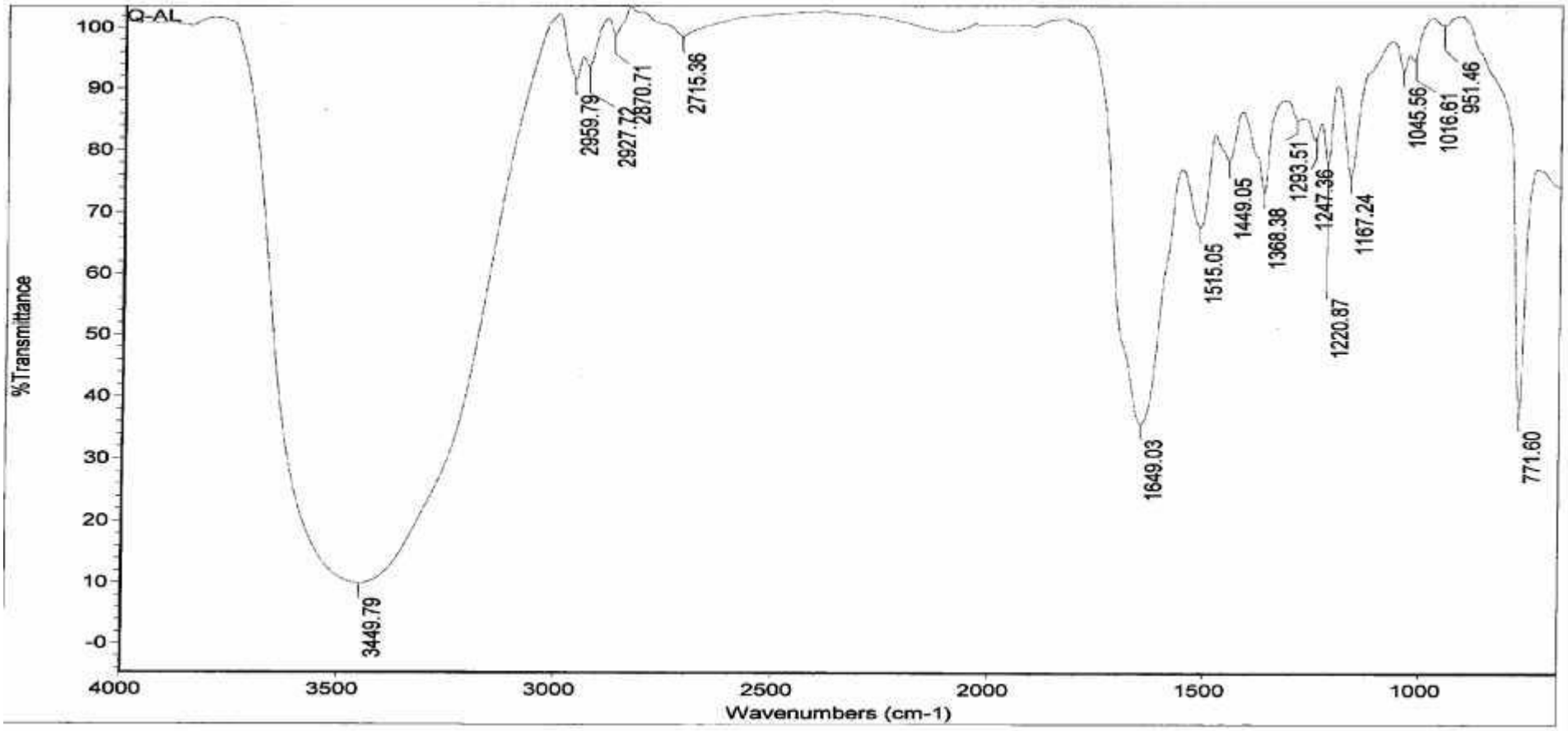
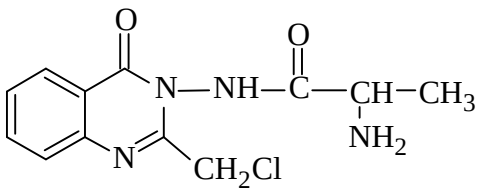


Figure 3: IR Spectra of QAL

Interpretation of IR spectra of QAA



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-6

S. No	Wave numbers (cm^{-1})	Functional groups
1	3435	NH Stretching (NH_2)
2	2978	Aromatic –CH- Stretching
3	1697	C=O Stretching in amide
4	1585	C=N Stretching
5	1296	C-N Stretching
6	667	C-Cl Stretching

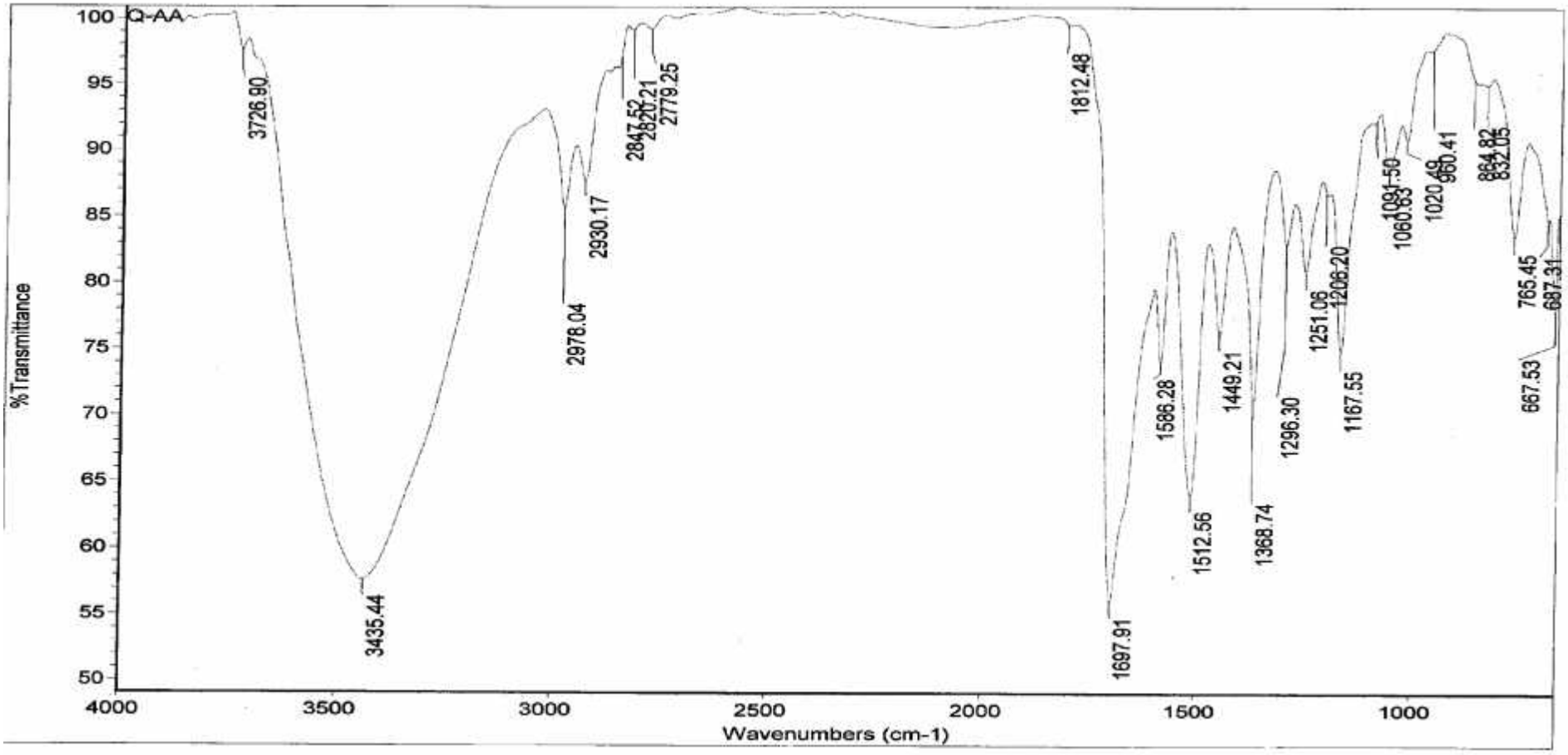
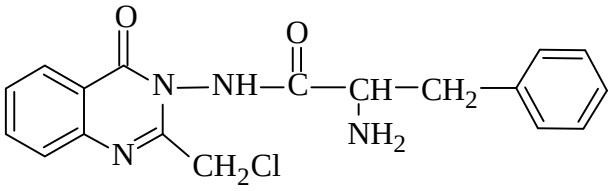


Figure 4: IR Spectra of QAA

Interpretation of IR spectra of QAPA



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-7

S. No	Wave numbers (cm^{-1})	Functional groups
1	3434	NH Stretching (NH_2)
2	1524	NH- Stretching
3	1683	C=O Stretching in amide
4	1524	C=N Stretching
5	1299	C-N Stretching
6	700	C-Cl Stretching

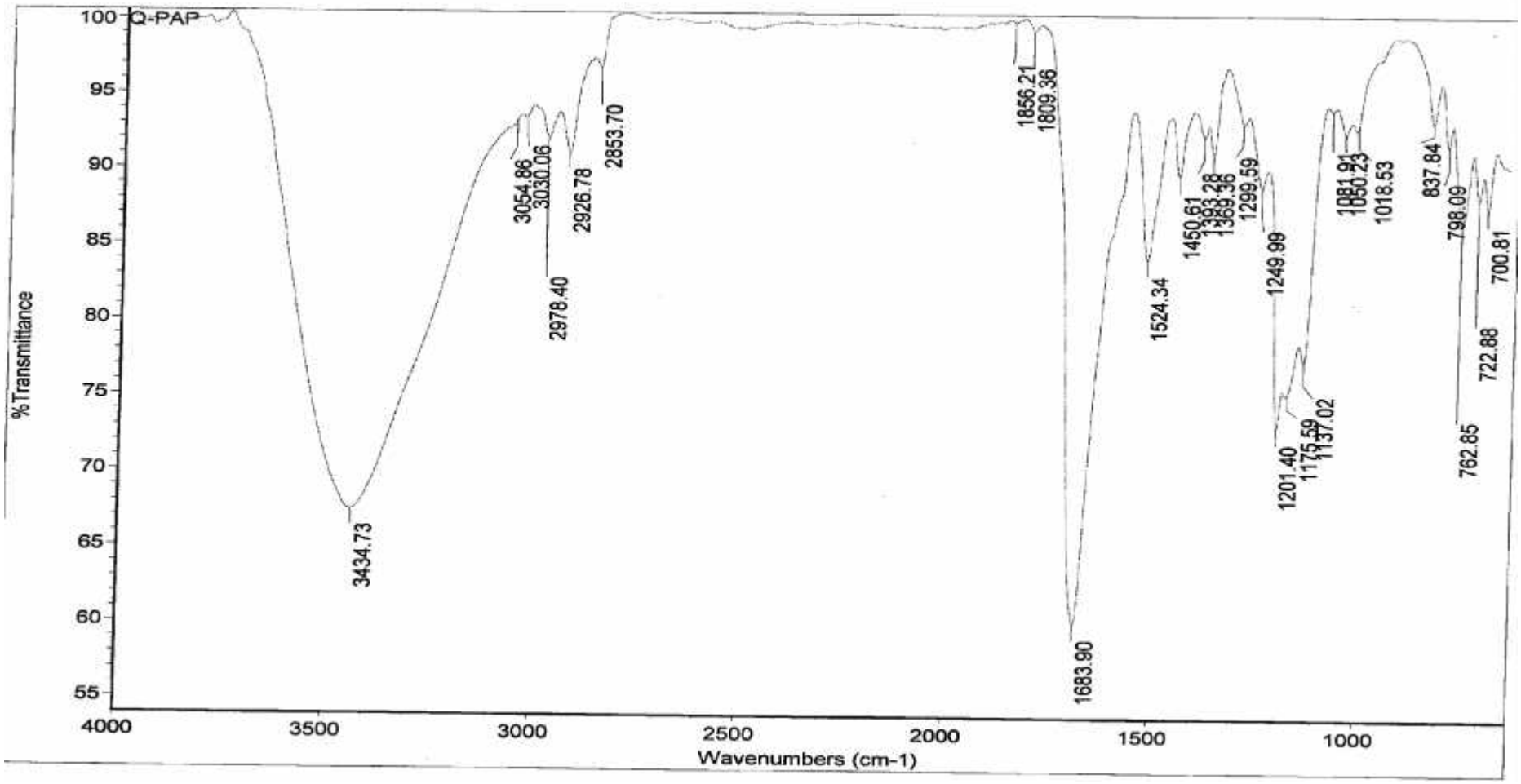
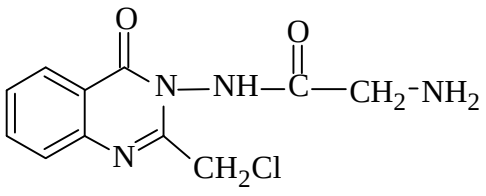


Figure 5: IR Spectra of QAPA

Interpretation of IR spectra of QAG



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-8

S. No	Wave numbers (cm^{-1})	Functional groups
1	3431	NH Stretching (NH_2)
2	1524	NH- Stretching
3	1686	C=O Stretching in amide
4	1585	C=N Stretching
5	1289	C-N Stretching
6	771	C-Cl Stretching

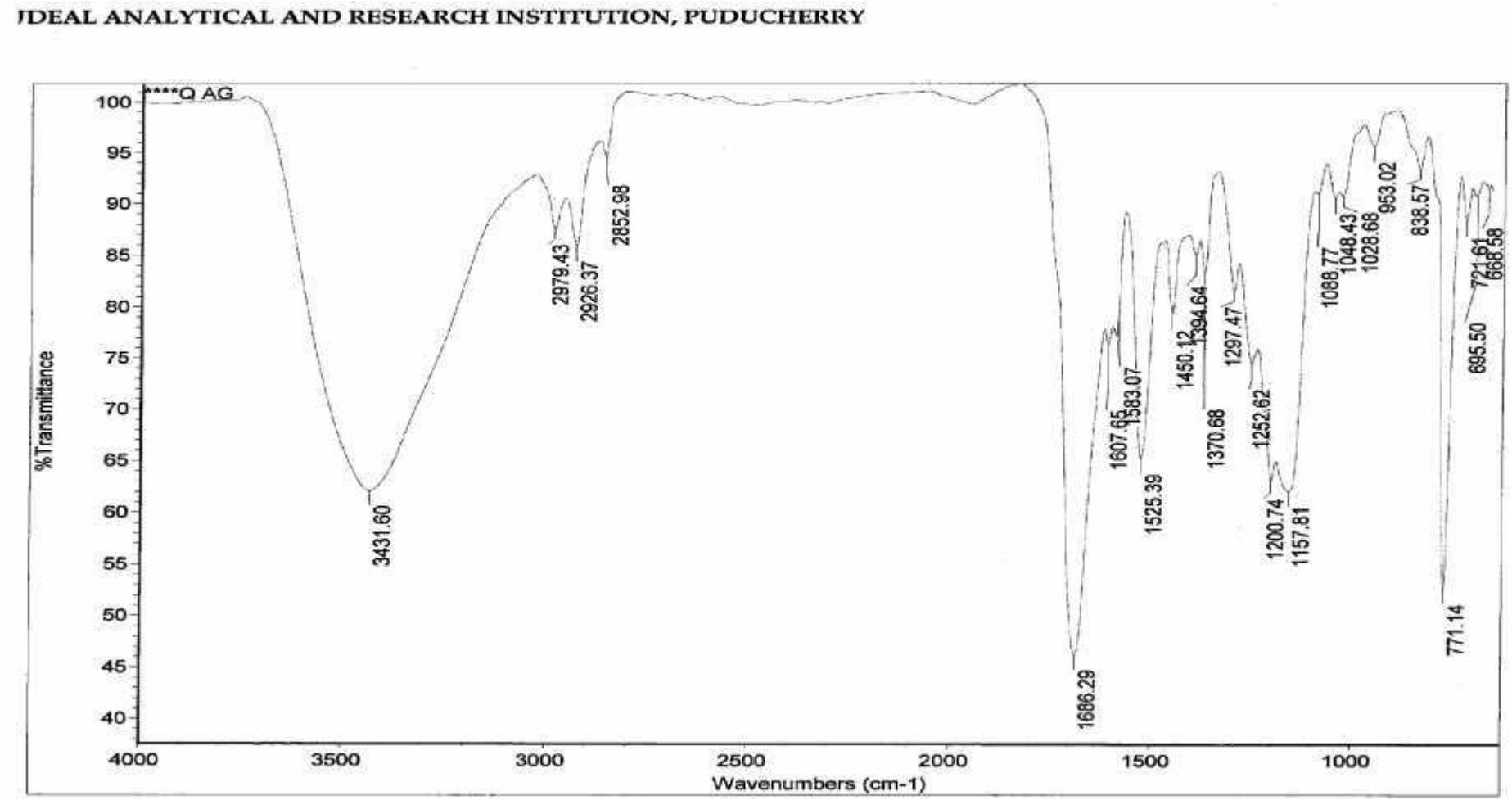
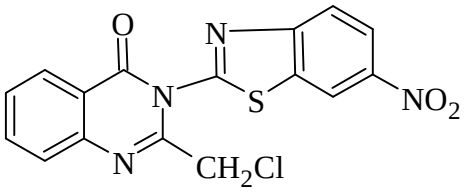


Figure 6: IR Spectra of QAG

Interpretation of IR spectra of QBEN



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-9

S. No	Wave numbers (cm^{-1})	Functional groups
1	3094	Aromatic-C-H Stretching
2	1647	C=O Stretching in amide
3	1570	C=N Stretching
4	1330	C-N Stretching
5	720	C-Cl Stretching
6	616	C-S bending

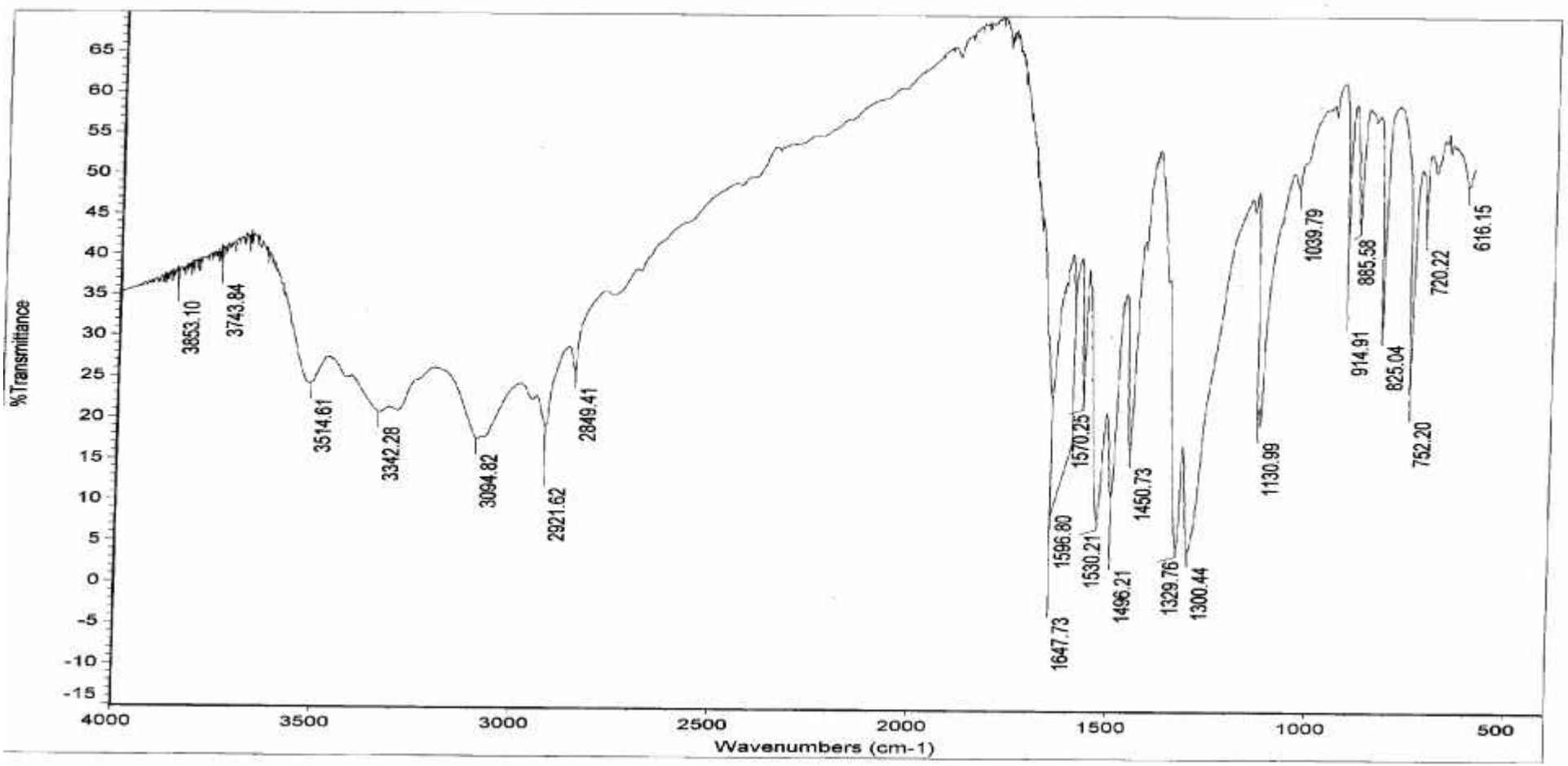
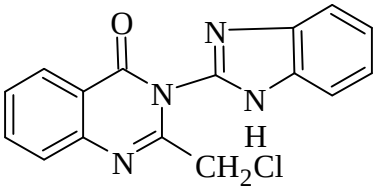


Figure 7: IR Spectra of QBEN

Interpretation of IR spectra of QIBM



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-10

S. No	Wave numbers (cm^{-1})	Functional groups
1	3483	N-H Stretching
2	3132	C-H Aromatic stretching
3	3055	Imidazole
4	1666	C=O Stretching in amide
5	1570	C=N Stretching
6	1566	C=C stretching
7	1313	C-N stretching
8	697	C-Cl stretching

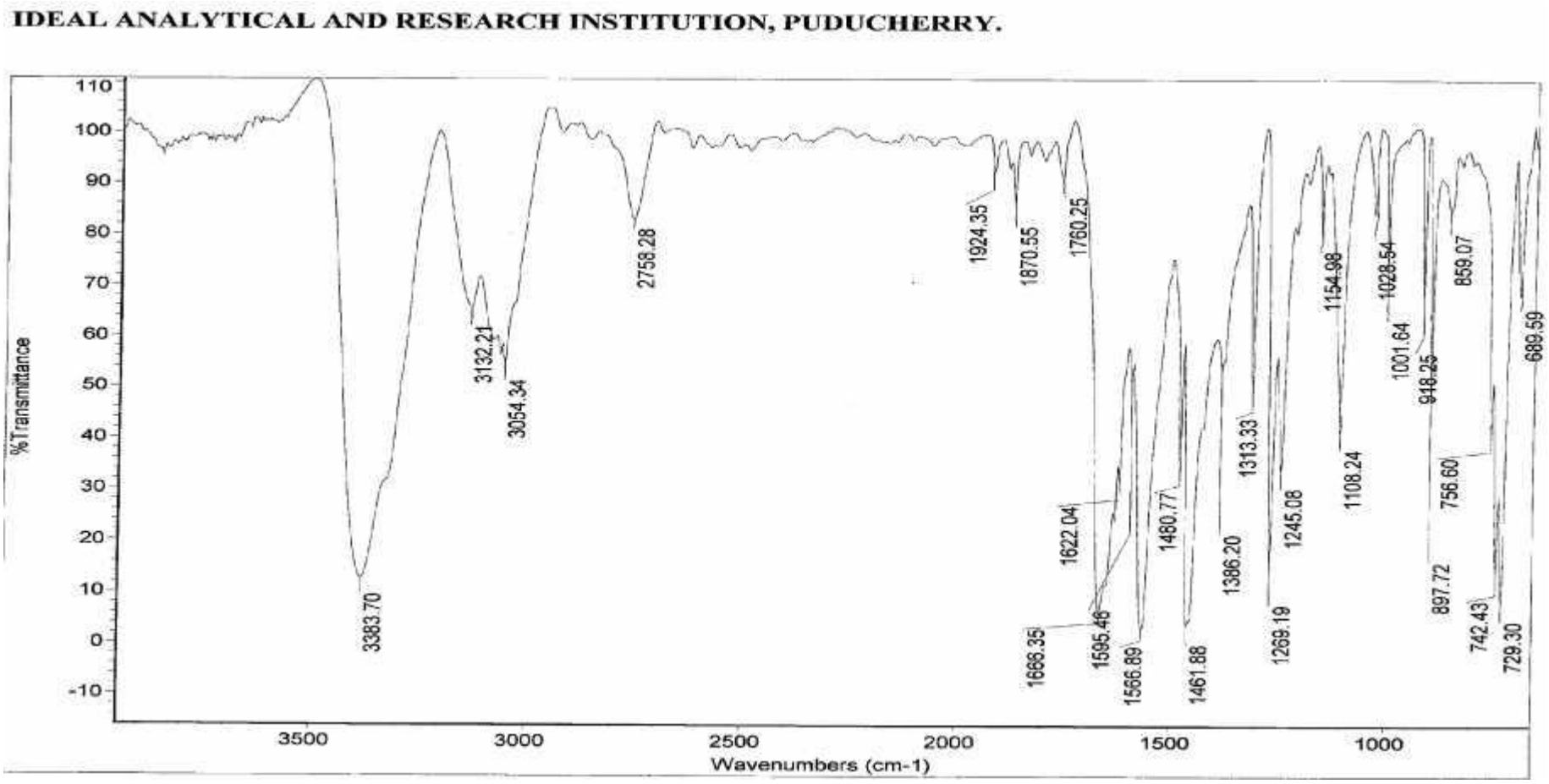
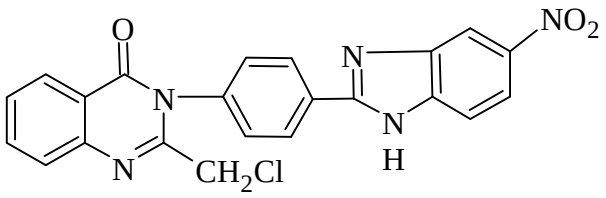


Figure 8: IR Spectra of QIBM

Interpretation of IR spectra of QNIBM



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-11

S. No	Wave numbers (cm^{-1})	Functional groups
1	3446	N-H Stretching
2	3102	C-H Aromatic stretching
3	1676	C=O Stretching in cyclic amide
4	1646	C=C Stretching
5	1528	C=N stretching
6	1343	C-N stretching
7	758	C-Cl stretching

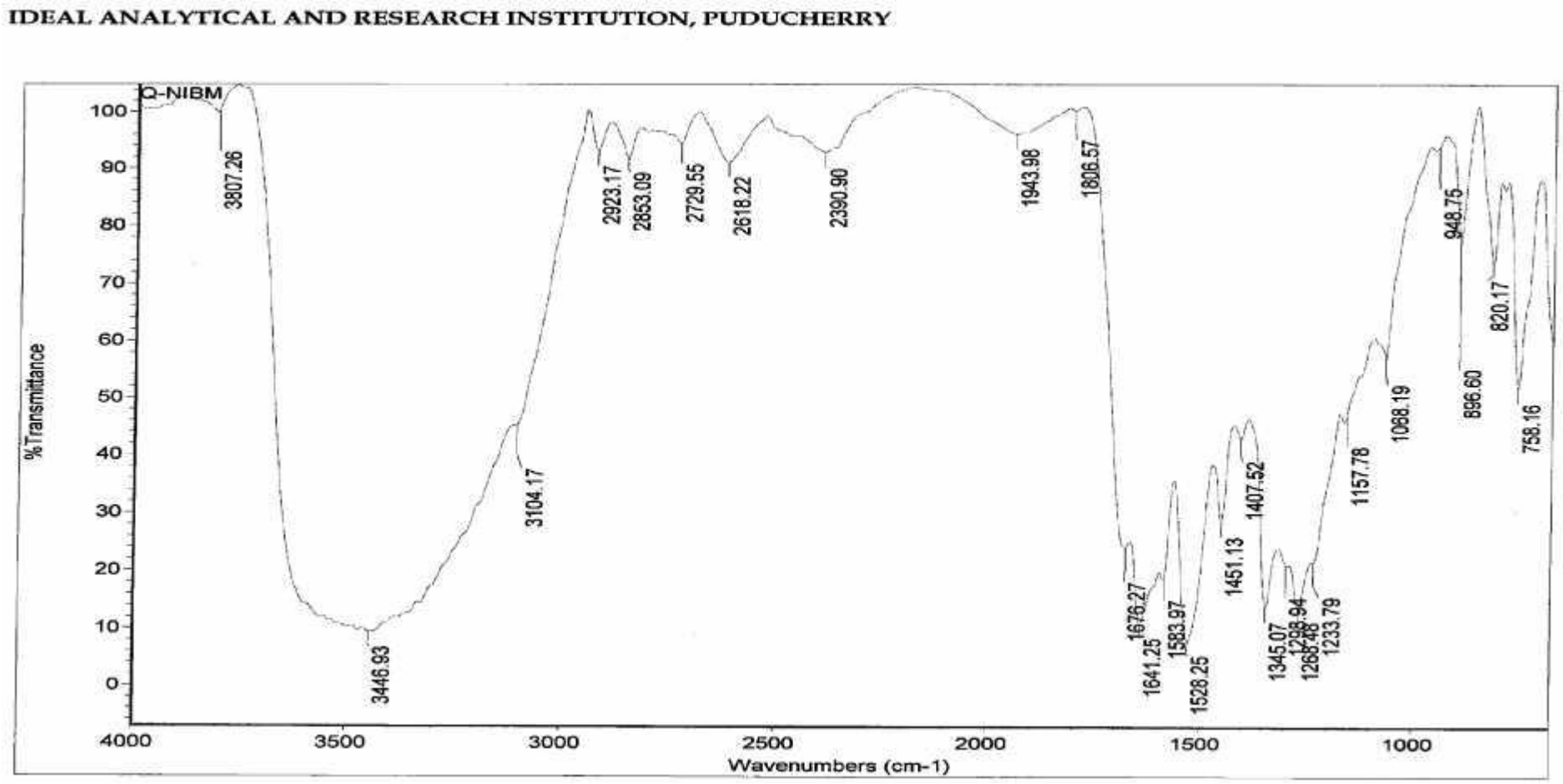
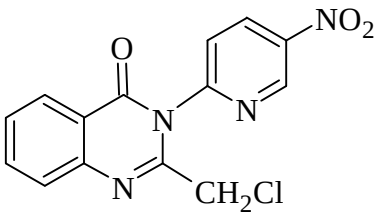


Figure 9: IR Spectra of QNIBM

Interpretation of IR spectra of QPIN



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-12

S. No	Wave numbers (cm^{-1})	Functional groups
1	3102	C-H Aromatic stretching
2	1676	C=O Stretching in cyclic amide
3	1606	C=C Stretching
4	1588	C=N stretching
5	1243	C-N stretching
6	1530	NO_2 Asymmetrical stretching
7	752	C-Cl stretching

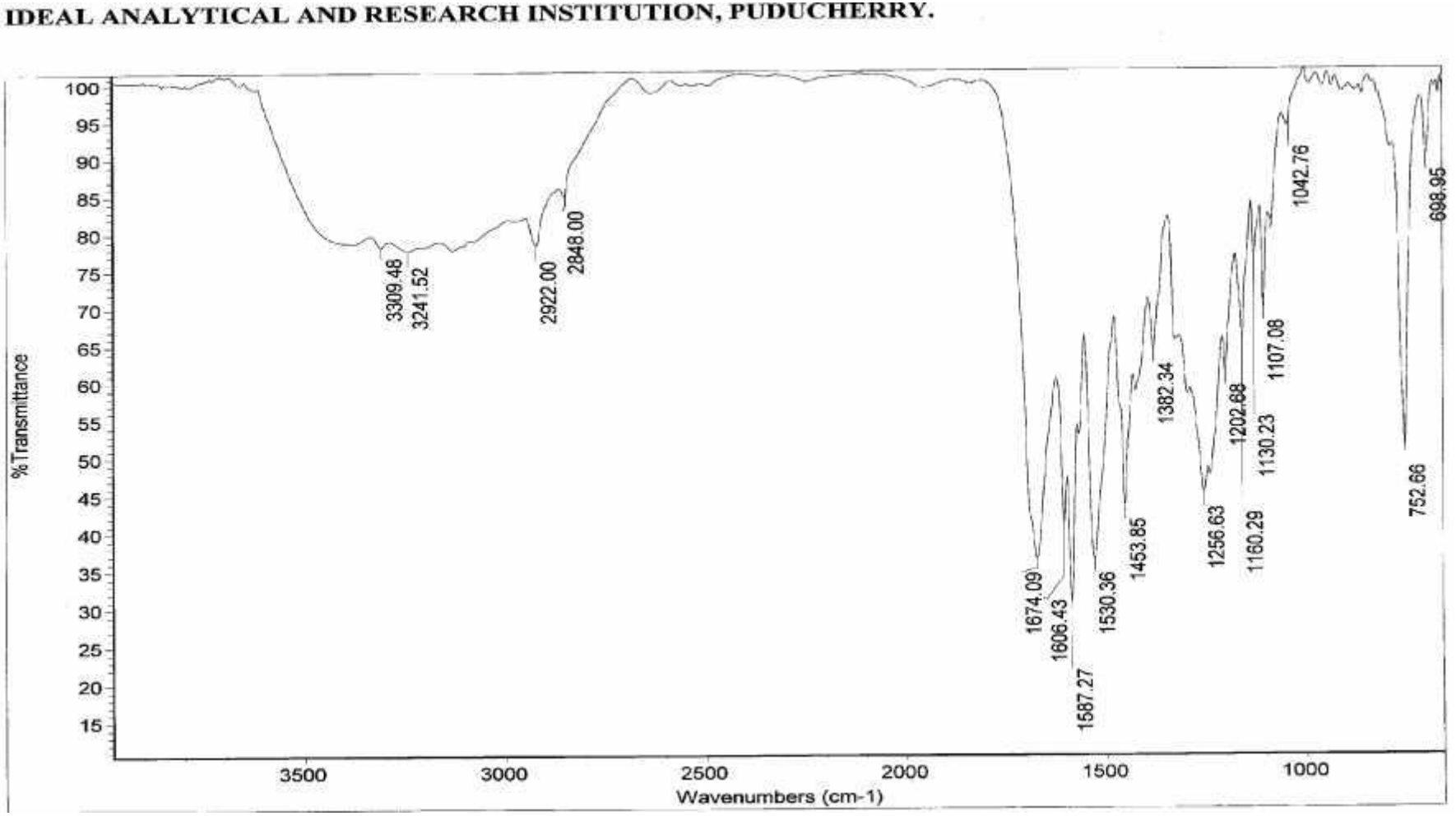
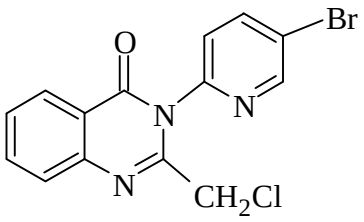


Figure 10: IR Spectra of QPIN

Interpretation of IR spectra of QBrP



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-13

S. No	Wave numbers (cm^{-1})	Functional groups
1	3242	C-H Aromatic stretching
2	1656	C=O Stretching in amide
3	1602	C=C Stretching
4	1586	C=N stretching
5	752	C-Cl stretching
6	650	C-Br bending

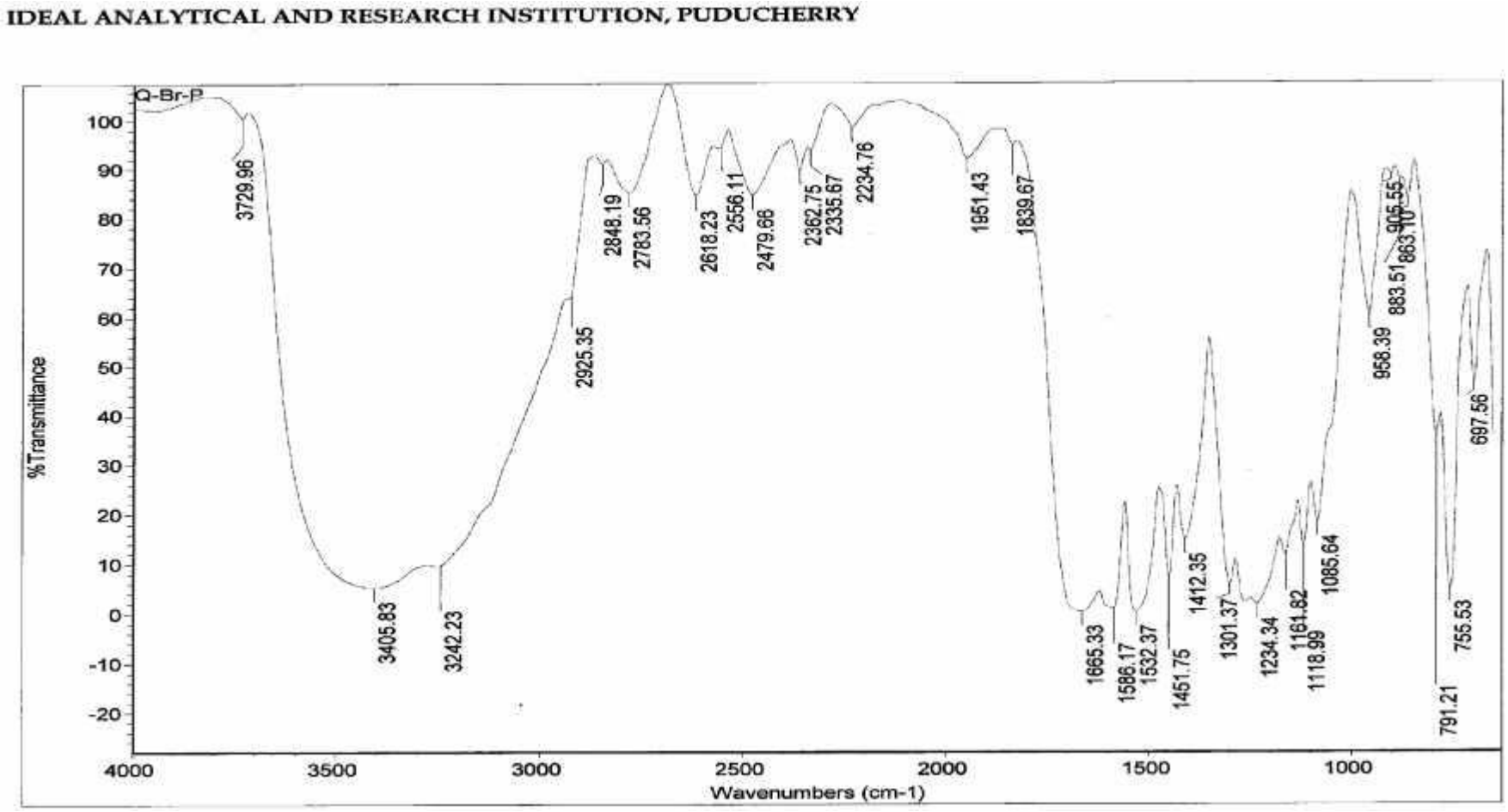
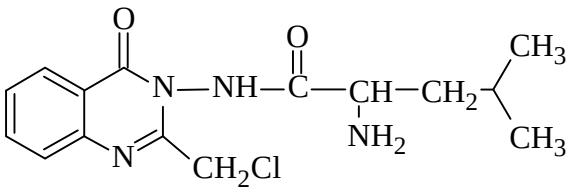


Figure 11: IR Spectra of QBrP

ii) NMR Spectral Analysis

The proton NMR values were recorded in the BRUKER 400 MHz NMR spectrophotometer

Interpretation of ^{1}H NMR spectra of QAL



The δ values with reference to the nature of protons are given below**.**

Table-14

S. No	Values in ppm	Nature of protons
1	8.1	Protons of aromatic ring in quinazolone
2	7.2	Protons in Amidic nitrogen
3	5.3	Protons in chloromethyl
4	1.5	Protons of primary amine
5	0.9	Proton of isopropyl group

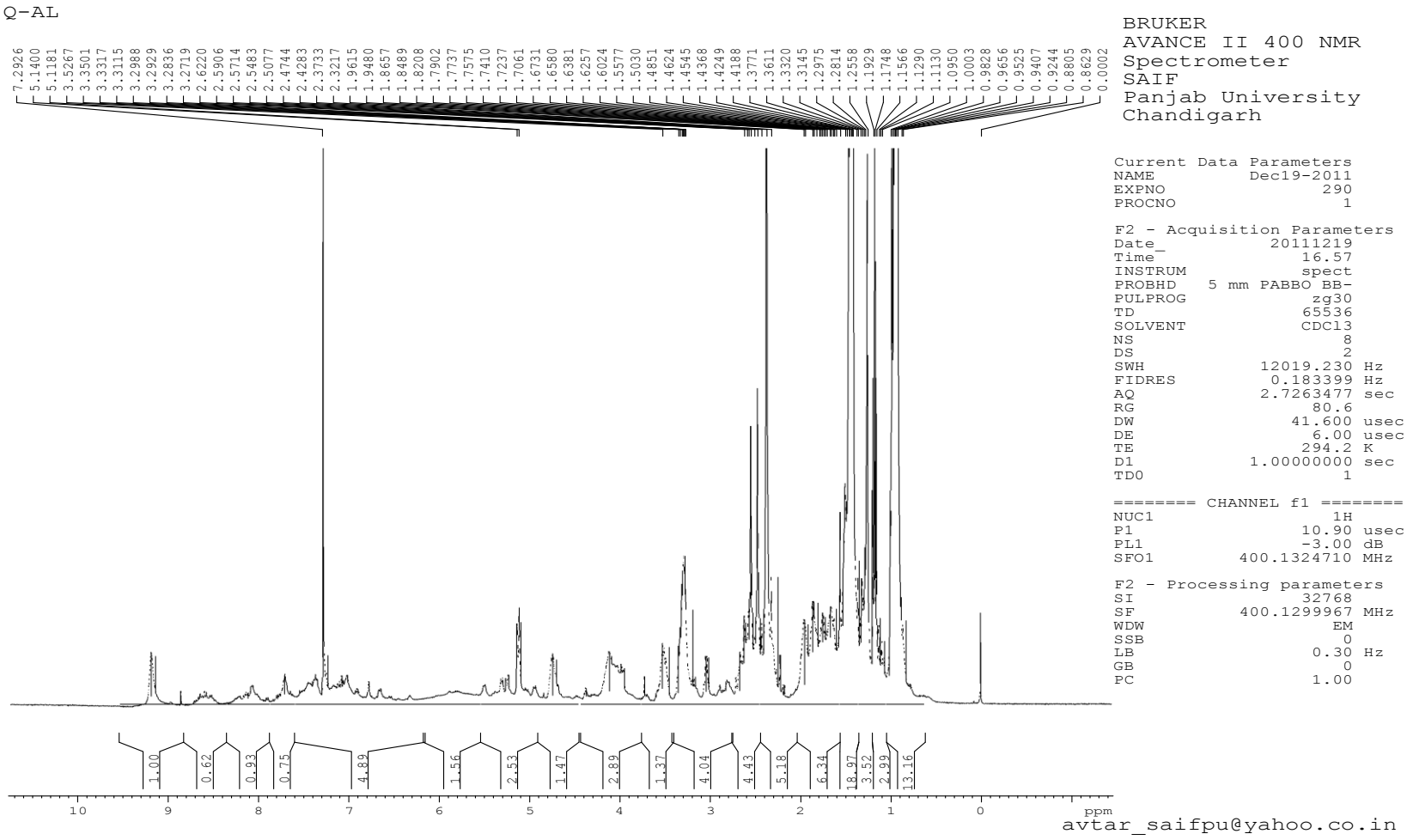


Figure 12: NMR Spectra of QAL

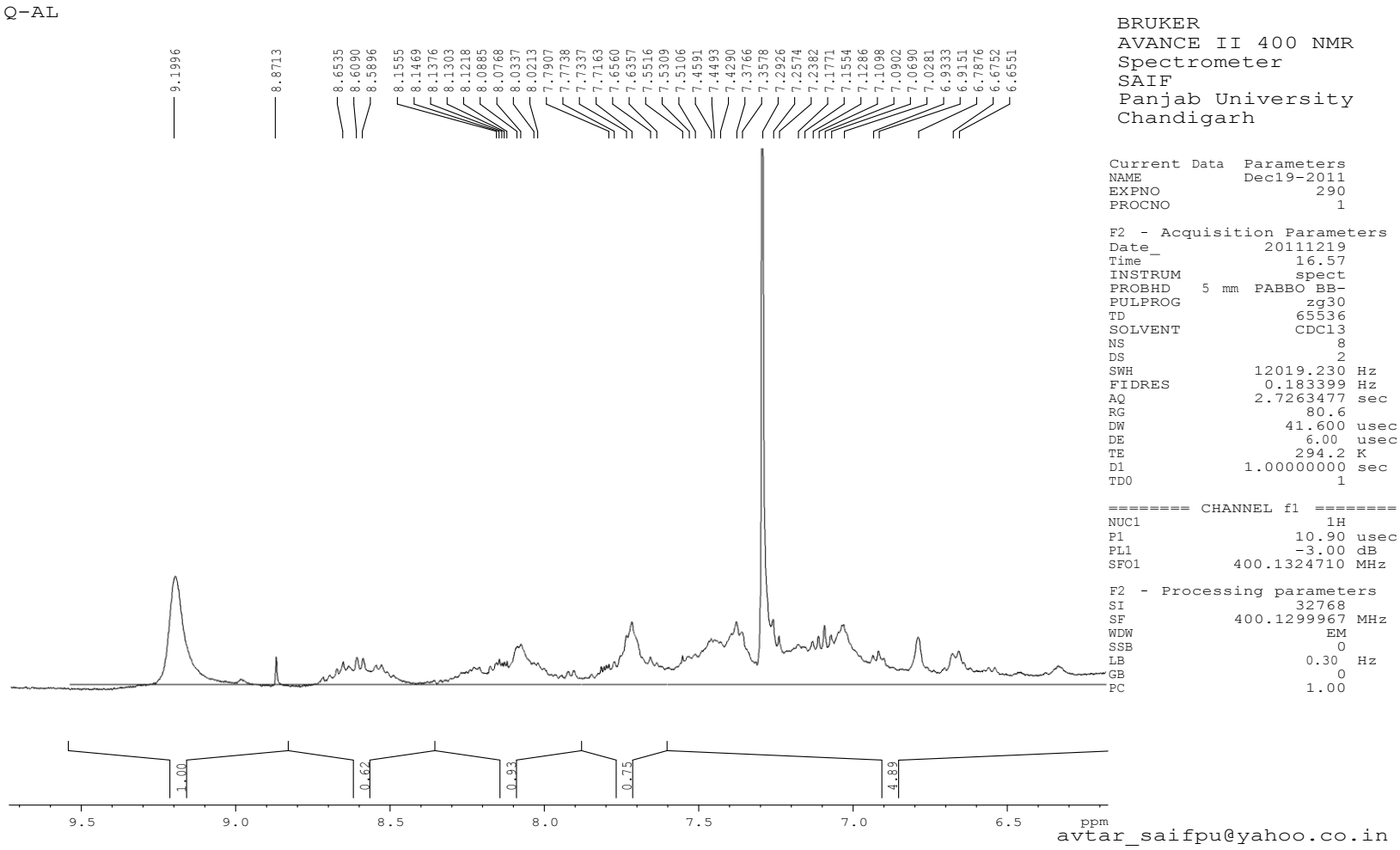


Figure 13: NMR Spectra of QAL

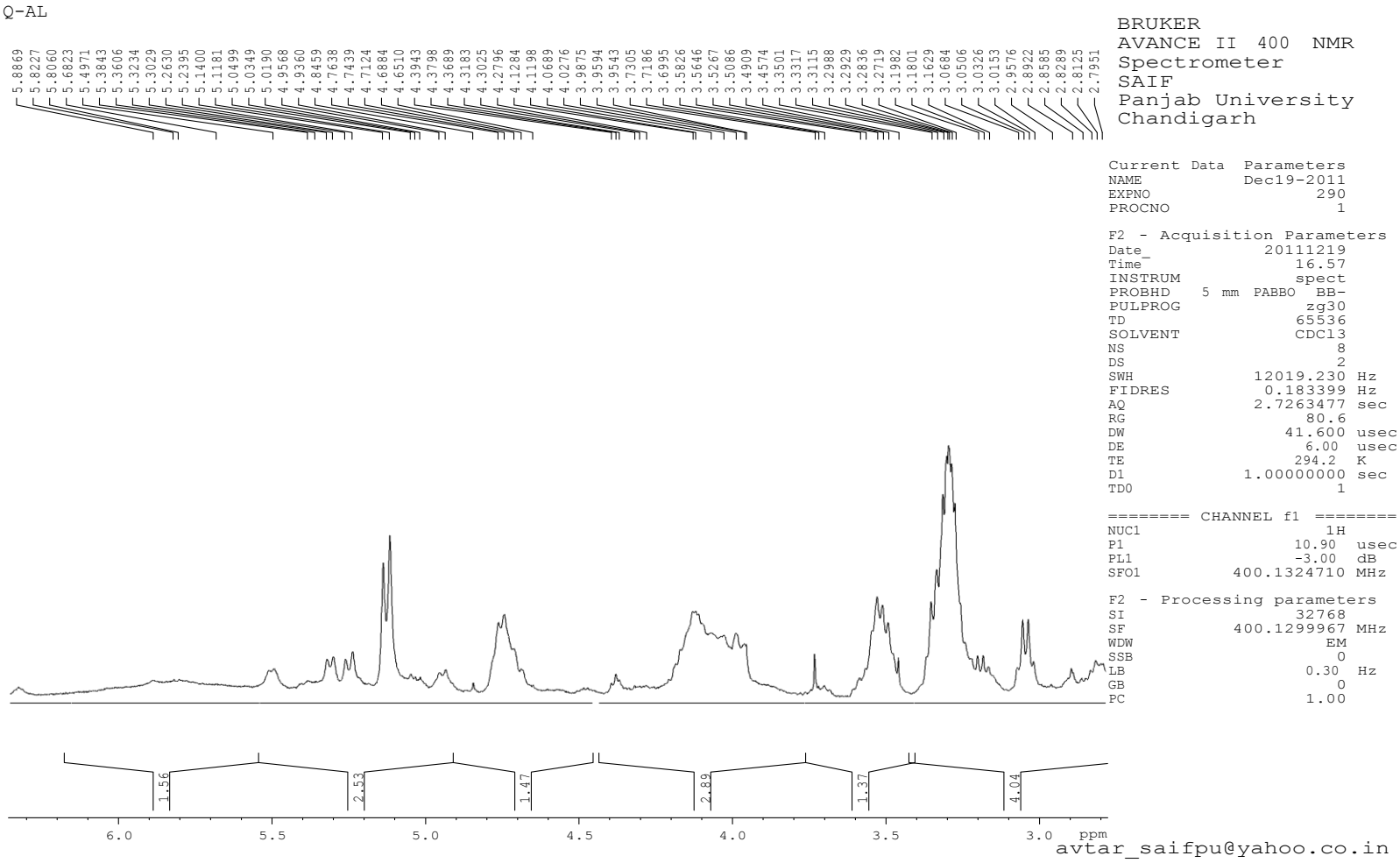


Figure 14: NMR Spectra of QAL

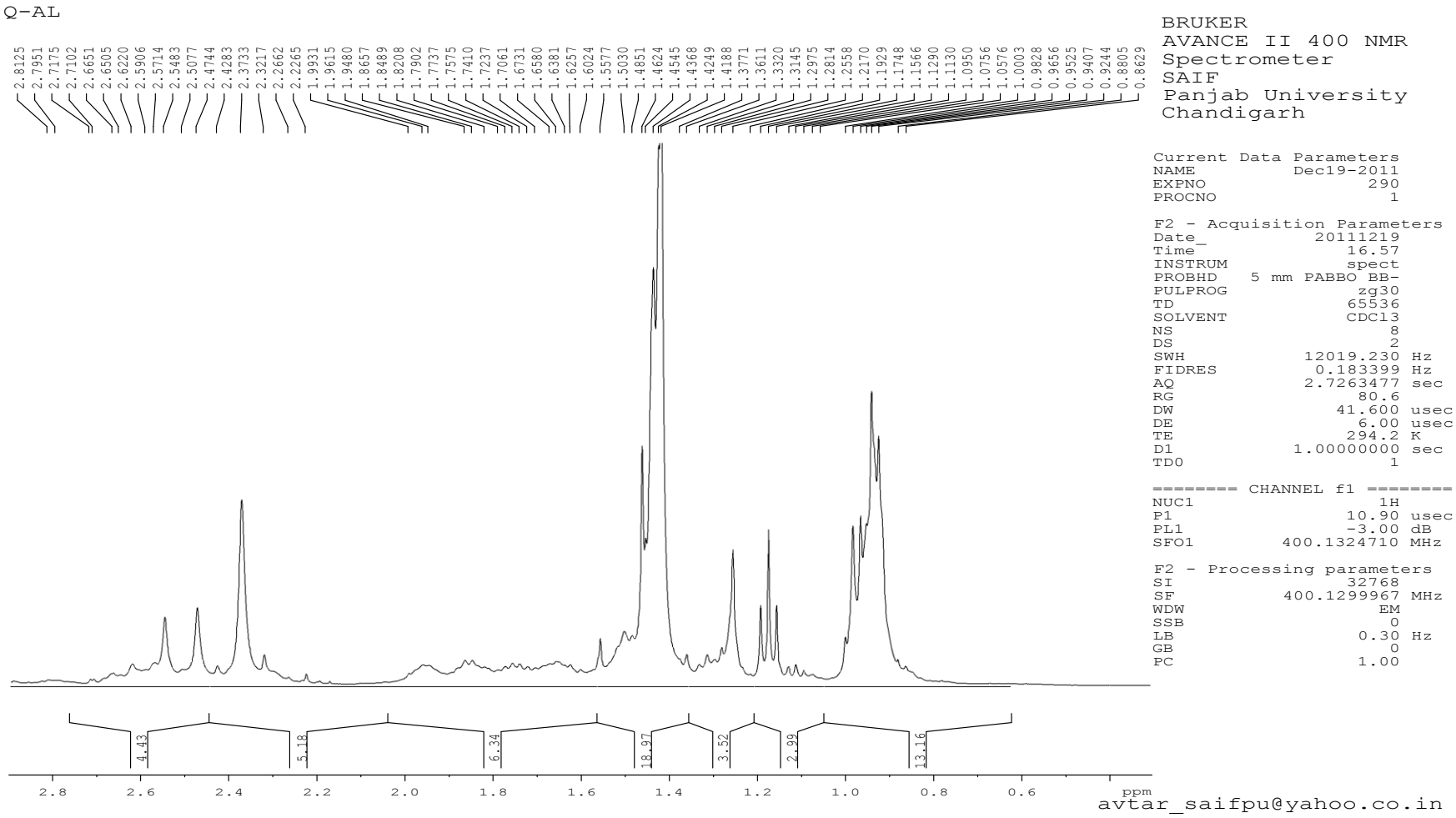


Figure 15: NMR Spectra of QAL

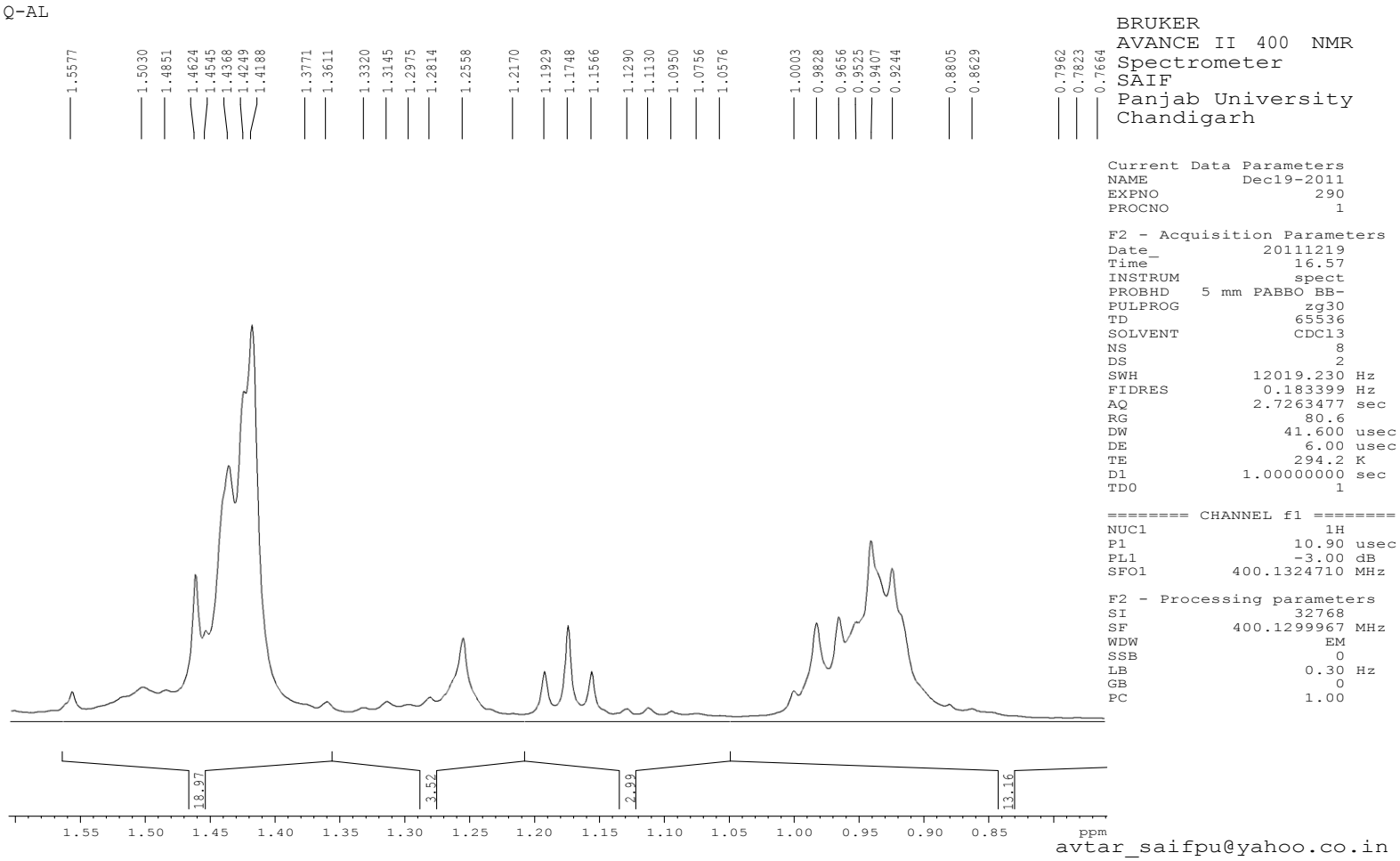
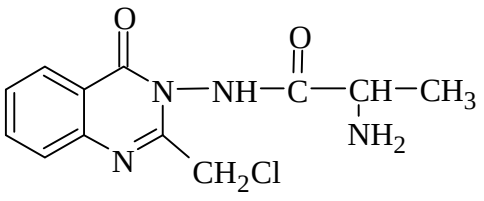


Figure 16: NMR Spectra of QAL

Interpretation of ^{1}H NMR spectra of QAA



The δ values with reference to the nature of protons are given below**.**

Table-15

S. No	Values in ppm	Nature of protons
1	8.1	Protons of aromatic ring in quinazolone
2	7.3	Protons of Amidic nitrogen
3	5.4	Protons in chloromethetyl
4	3.6	Alkyl proton of methine group
5	1.9	Protons of primary amine
6	1.3	Alkyl proton of methyl group
7	1.0	Proton of isopropyl group

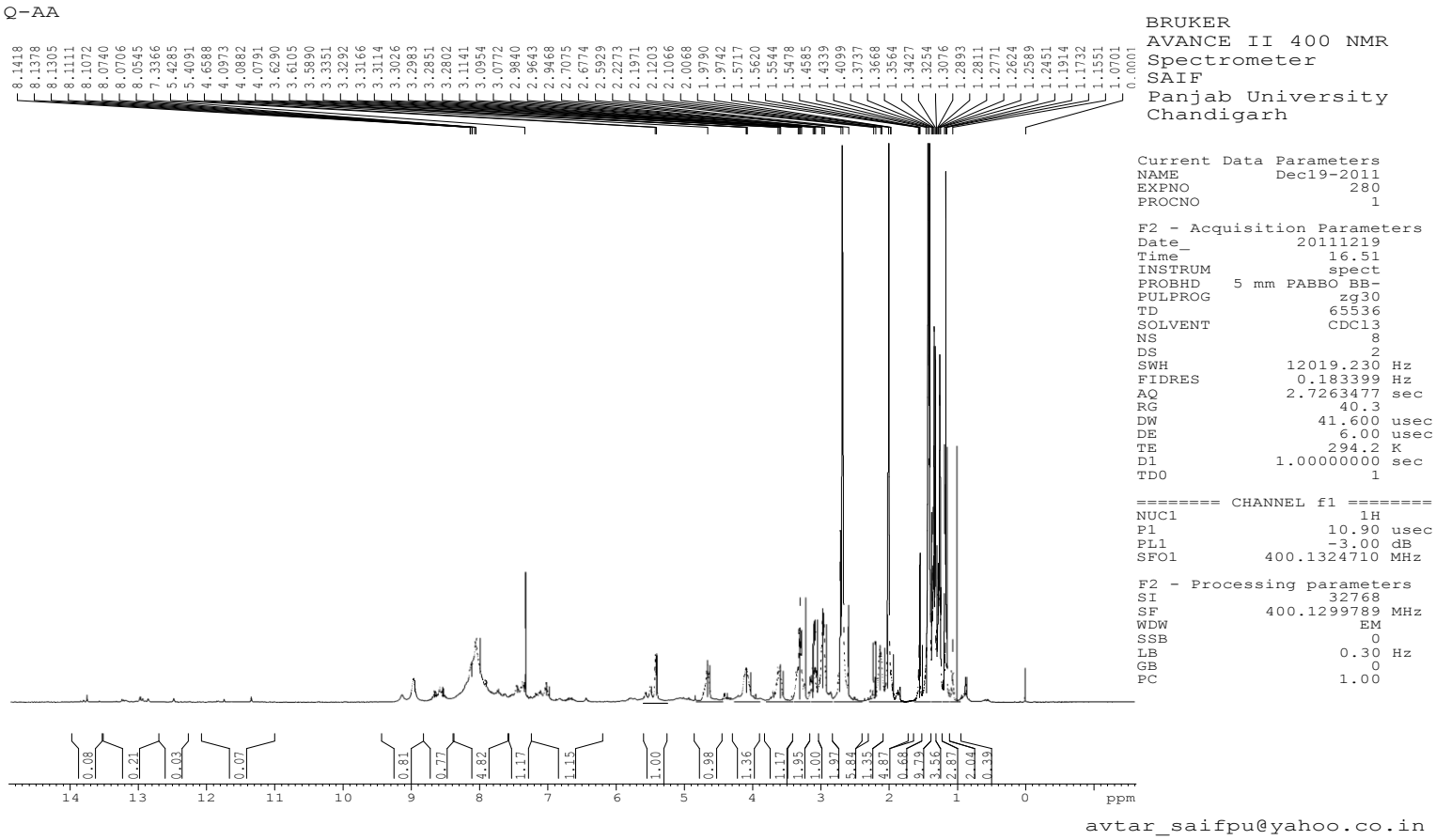


Figure 17: NMR Spectra of QAA

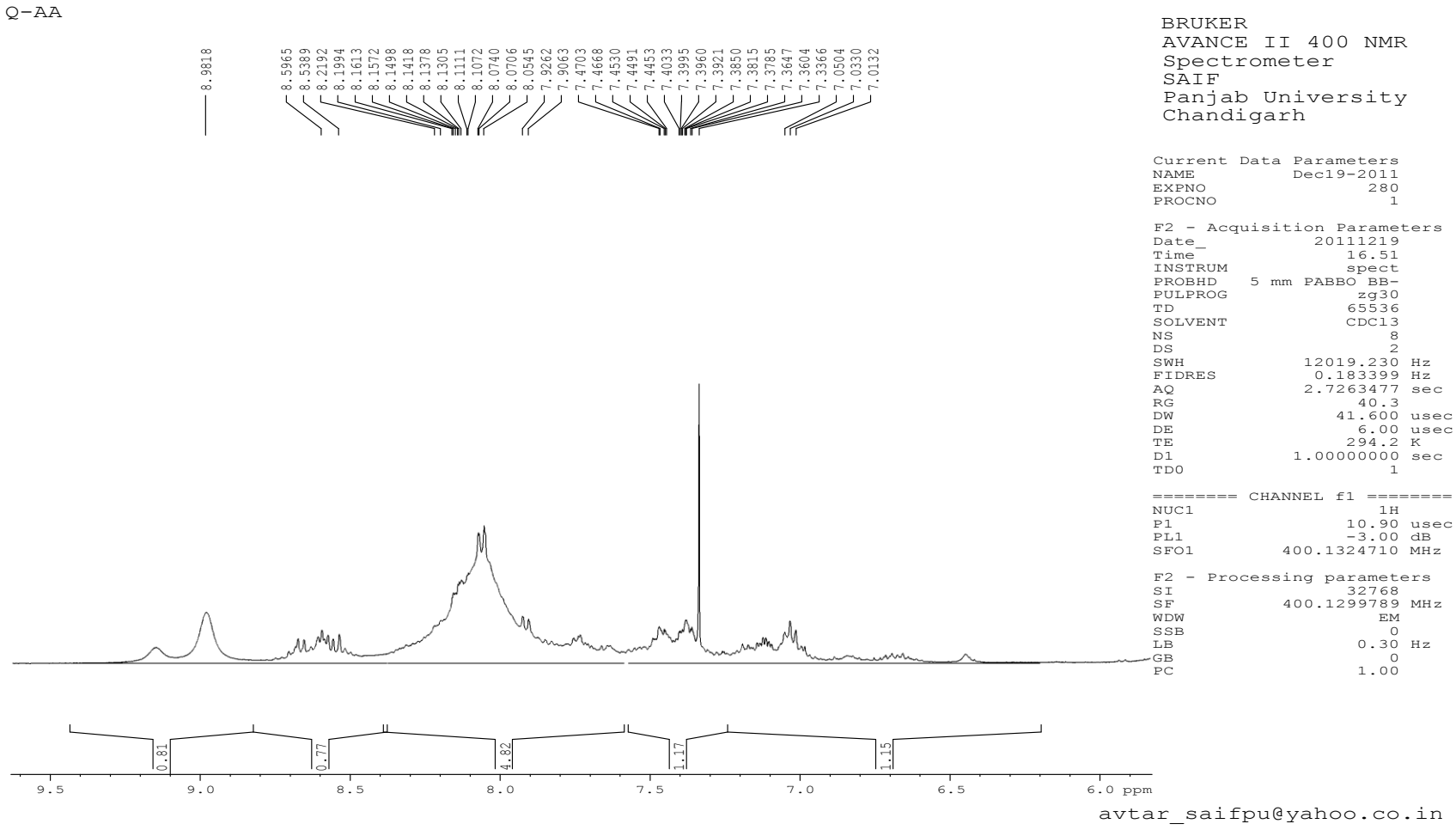


Figure 18: NMR Spectra of QAA

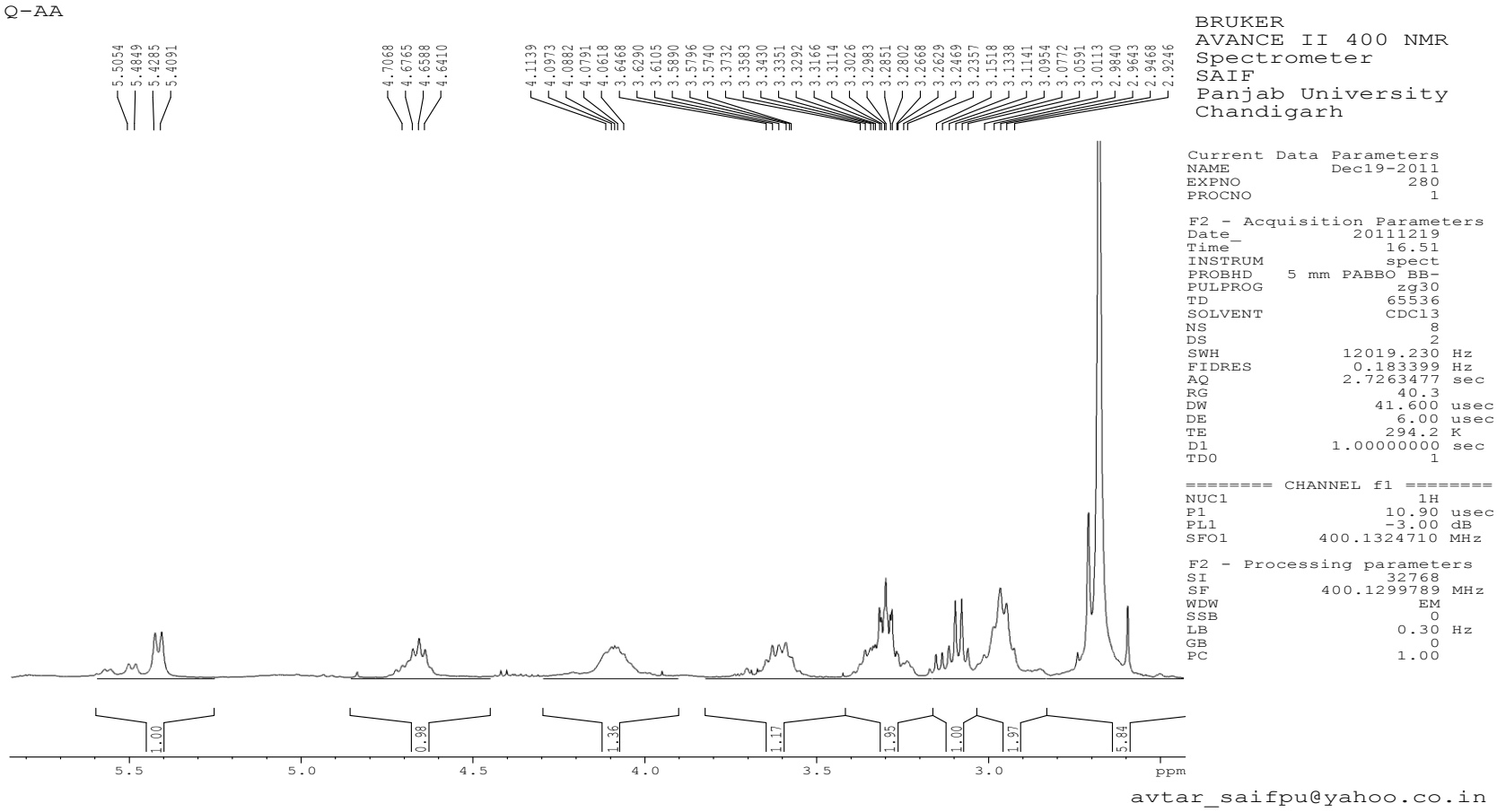
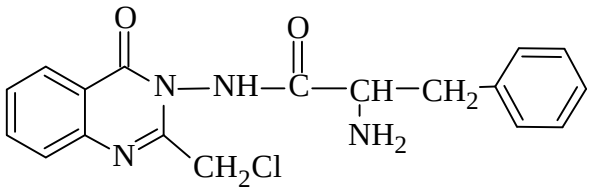


Figure 19: NMR Spectra of QAA

Interpretation of ^{1}H NMR spectra of QAPA



The δ values with reference to the nature of protons are given below**.**

Table-16

S. No	Values in ppm	Nature of protons
1	7.31	Protons of aromatic ring in quinazolone
2	7.18	Protons of free phenyl group in quinazolone
3	6.8	Protons of amidic nitrogen
4	5.1	Protons in chlorometheyl
5	1.7	Protons of primary amine
6	1.3	Alkyl proton of methyl group

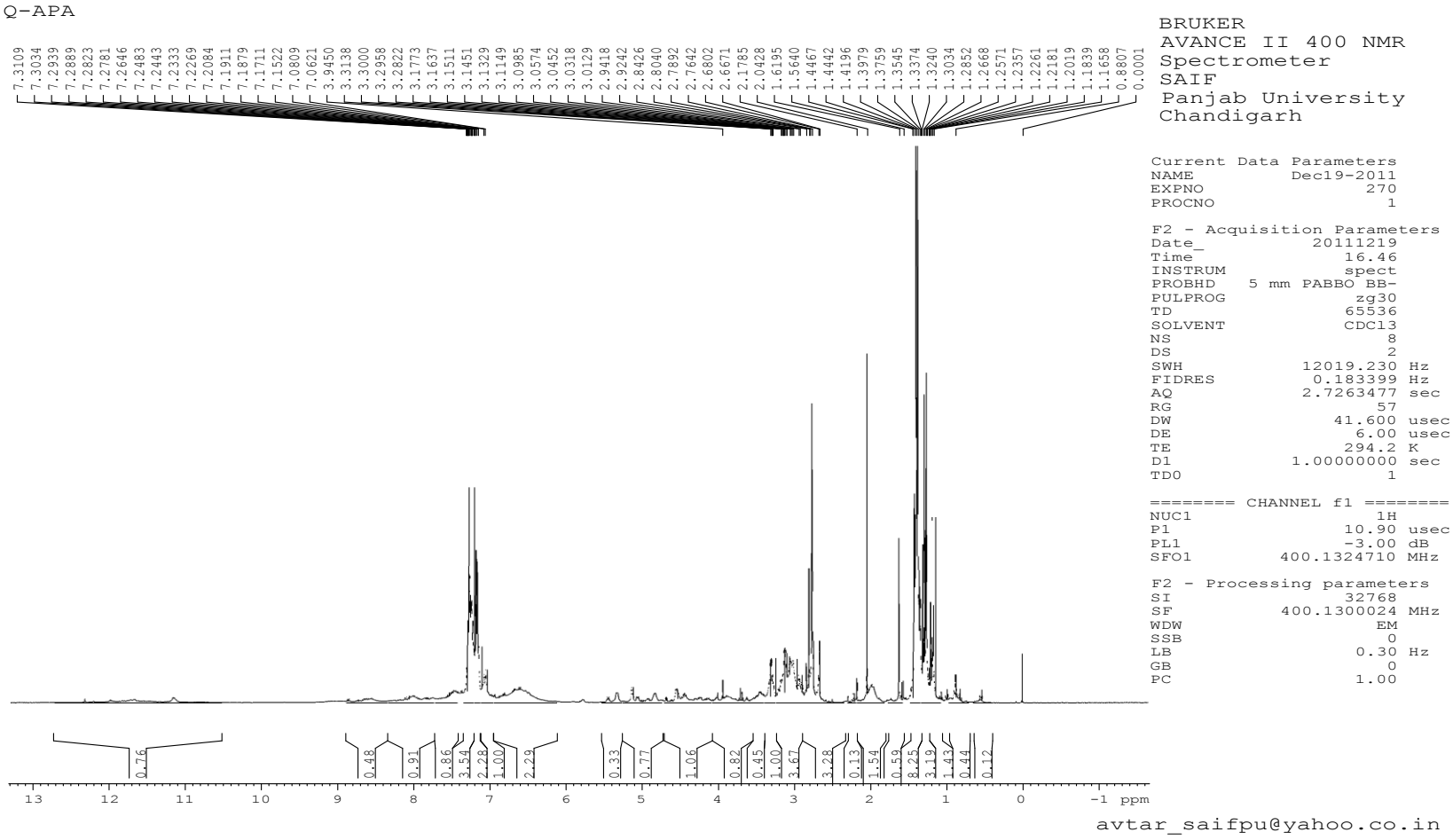


Figure 20: NMR Spectra of QAPA

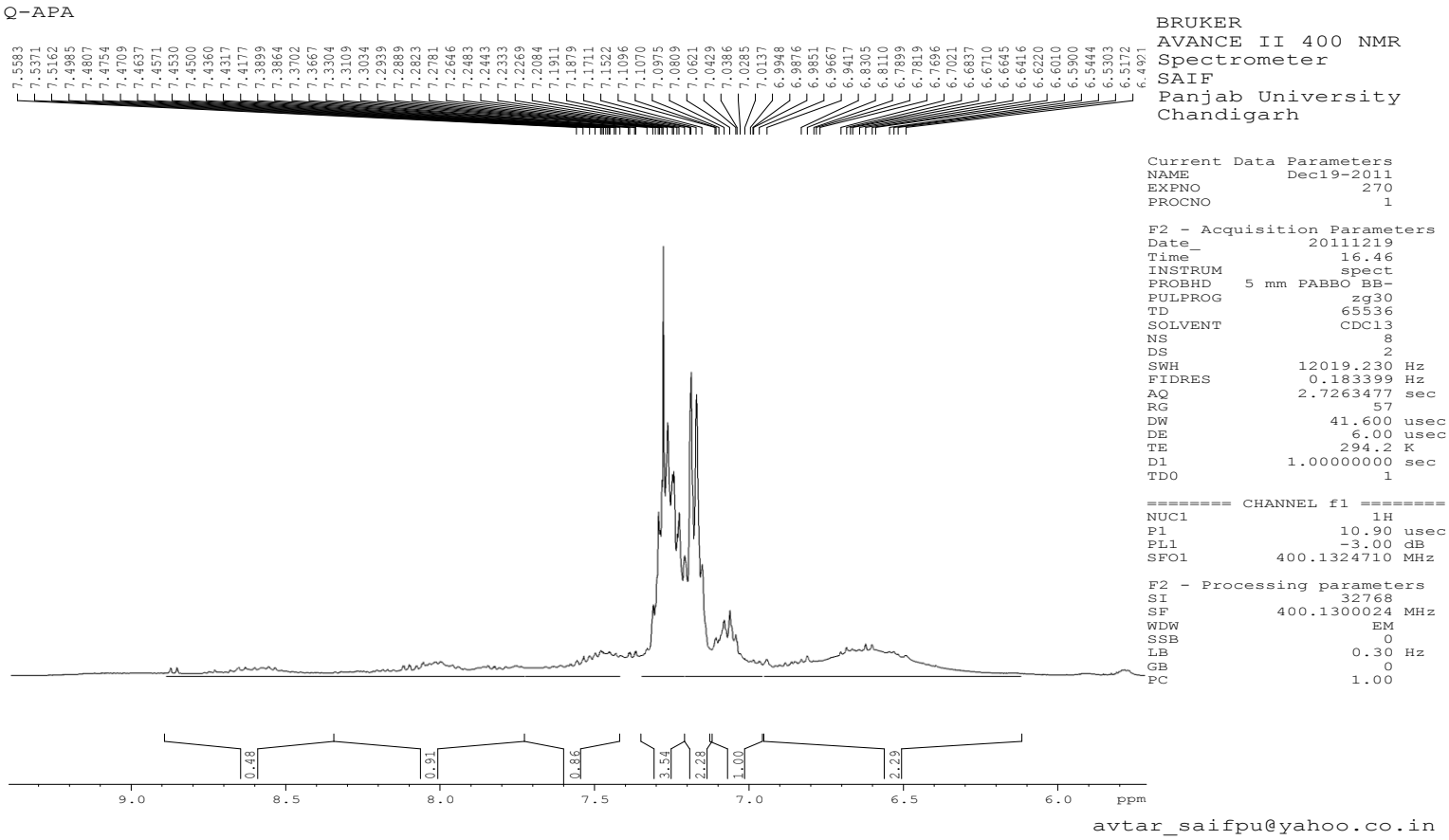


Figure 21: NMR Spectra of QAPA

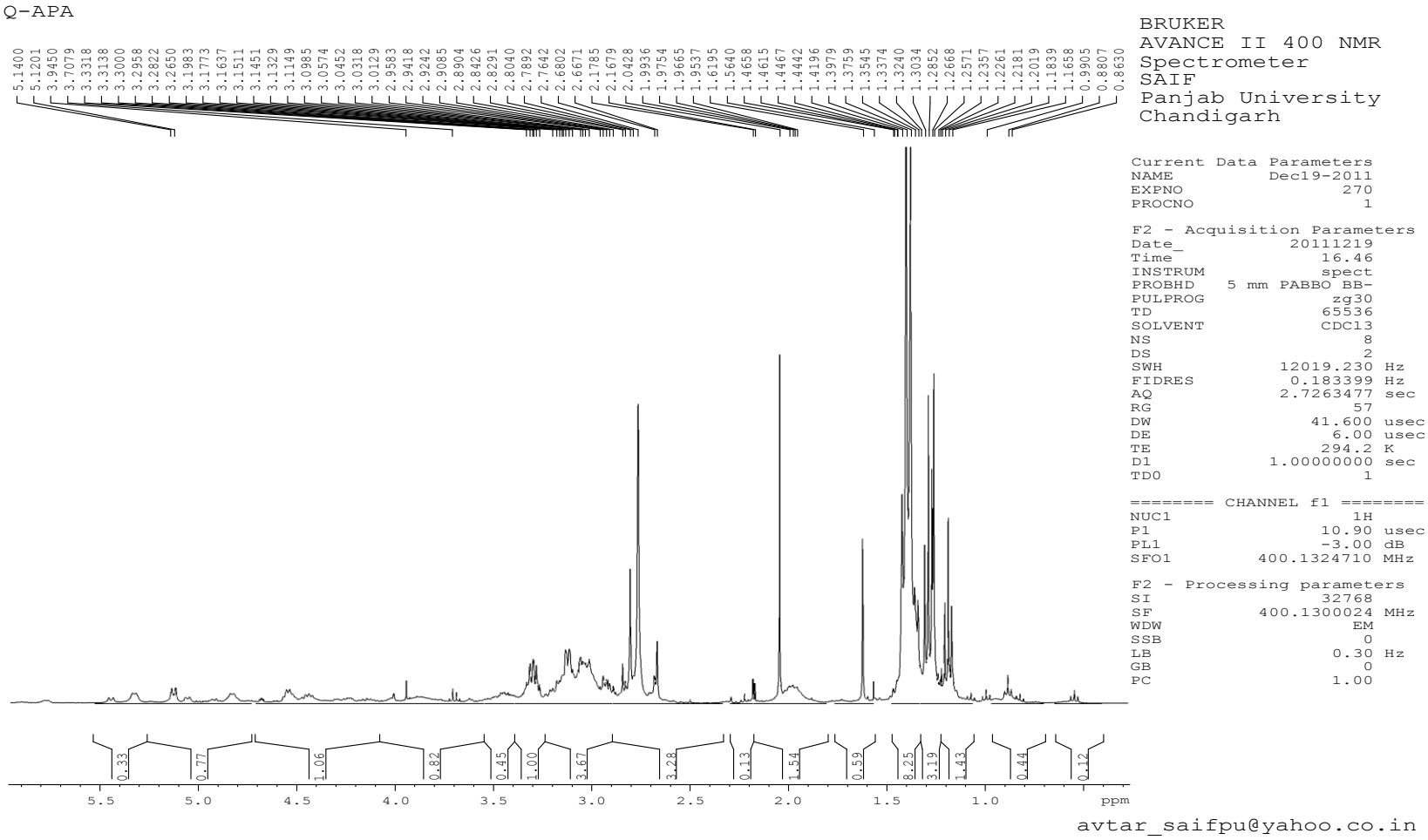


Figure 22: NMR Spectra of QAPA

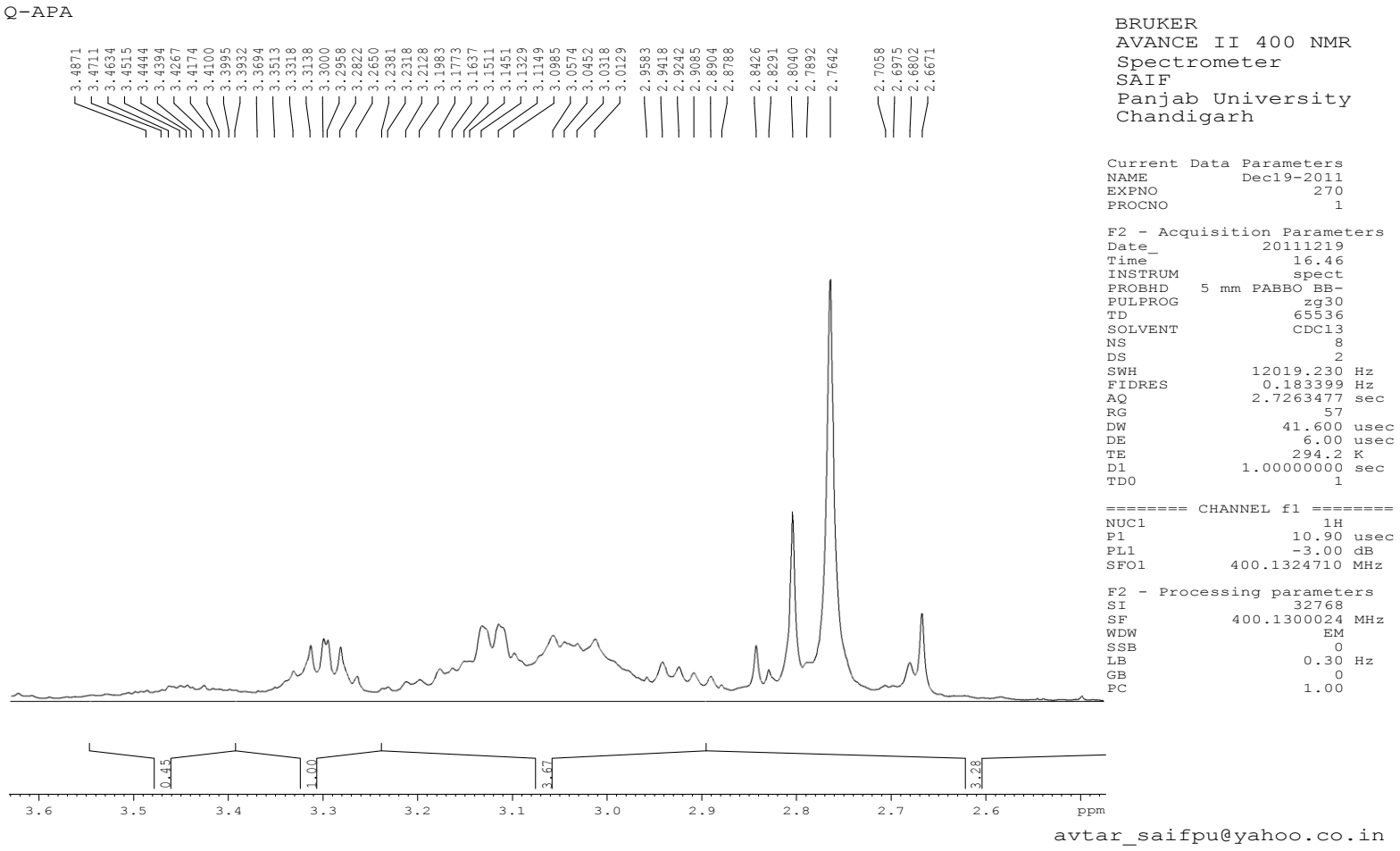


Figure 23: NMR Spectra of QAPA

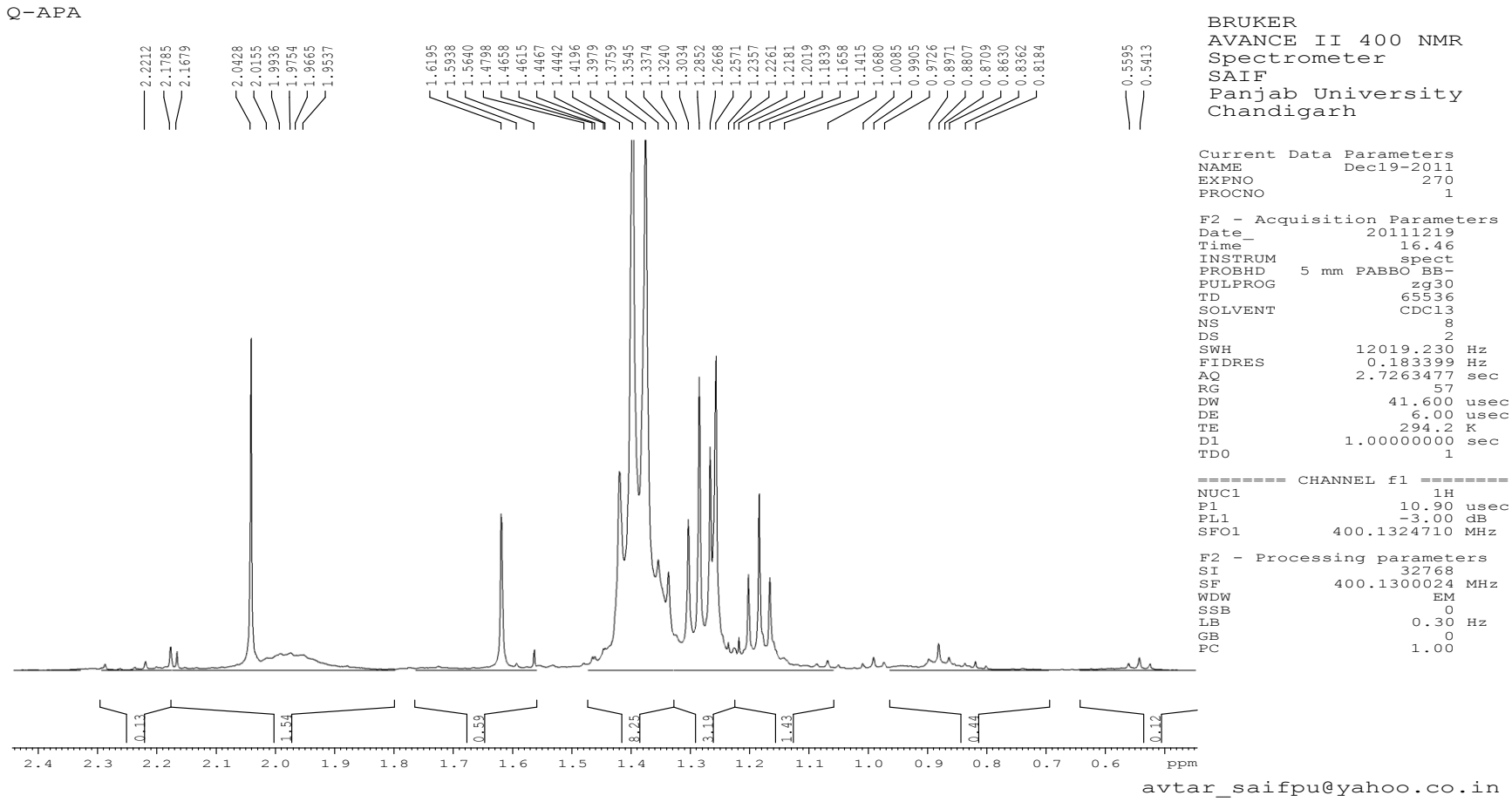
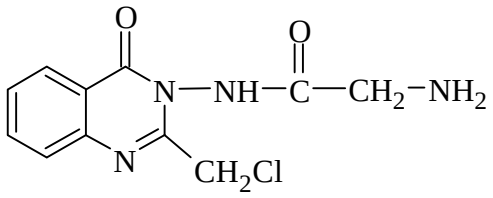


Figure 24: NMR Spectra of QAPA

Interpretation of ^{1}H NMR spectra of QAG



The δ values with reference to the nature of protons are given below**.**

Table-17

S. No	Values in ppm	Nature of protons
1	8.02	Protons of amidic nitrogen
2	7.4	Protons of aromatic ring in quinazolone
3	4.1-4.4	Protons in chloromethetheyl
4	3.8	proton of methyl group
5	1.5	Protons of primary amine

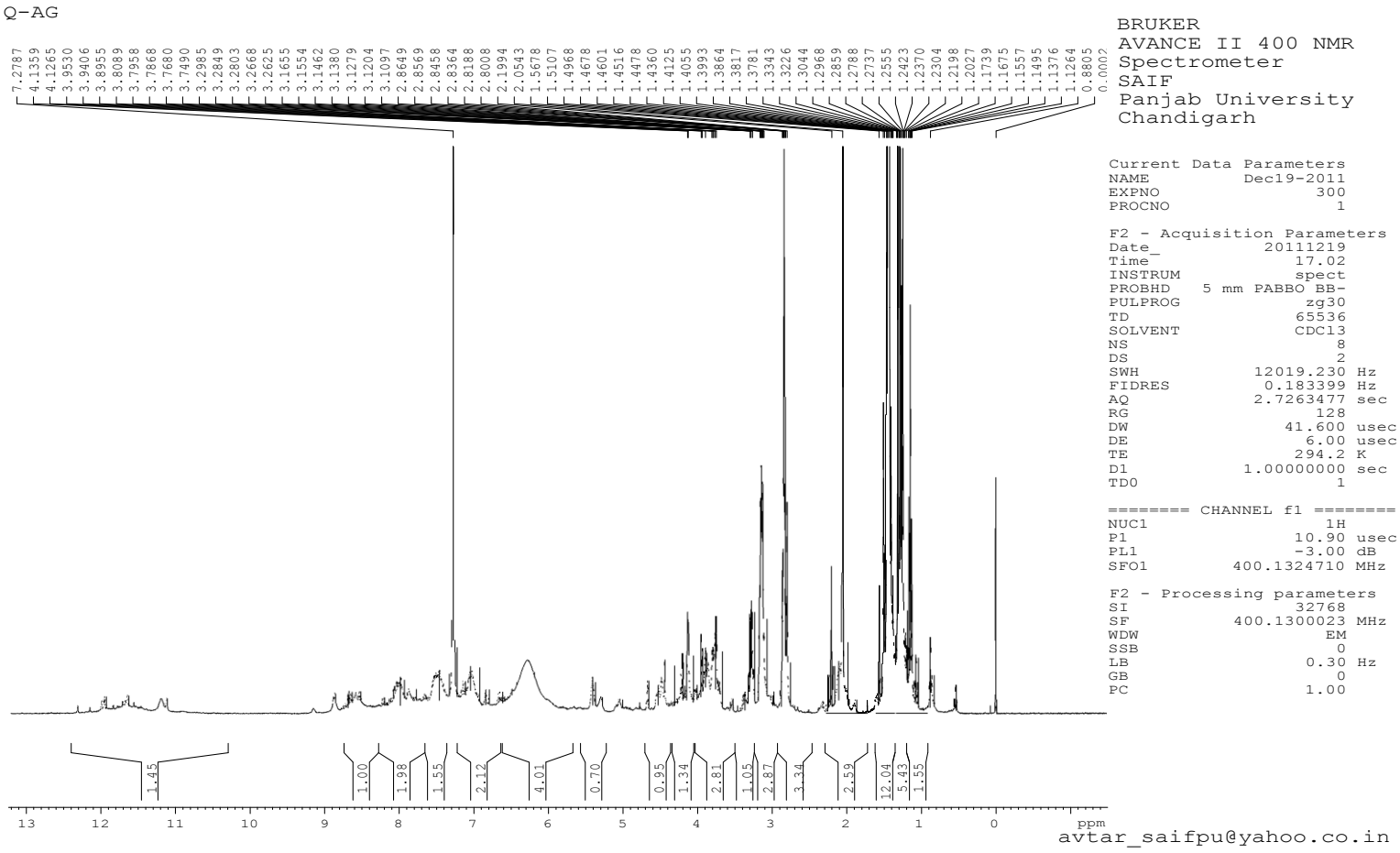


Figure 25: NMR Spectra of QAG

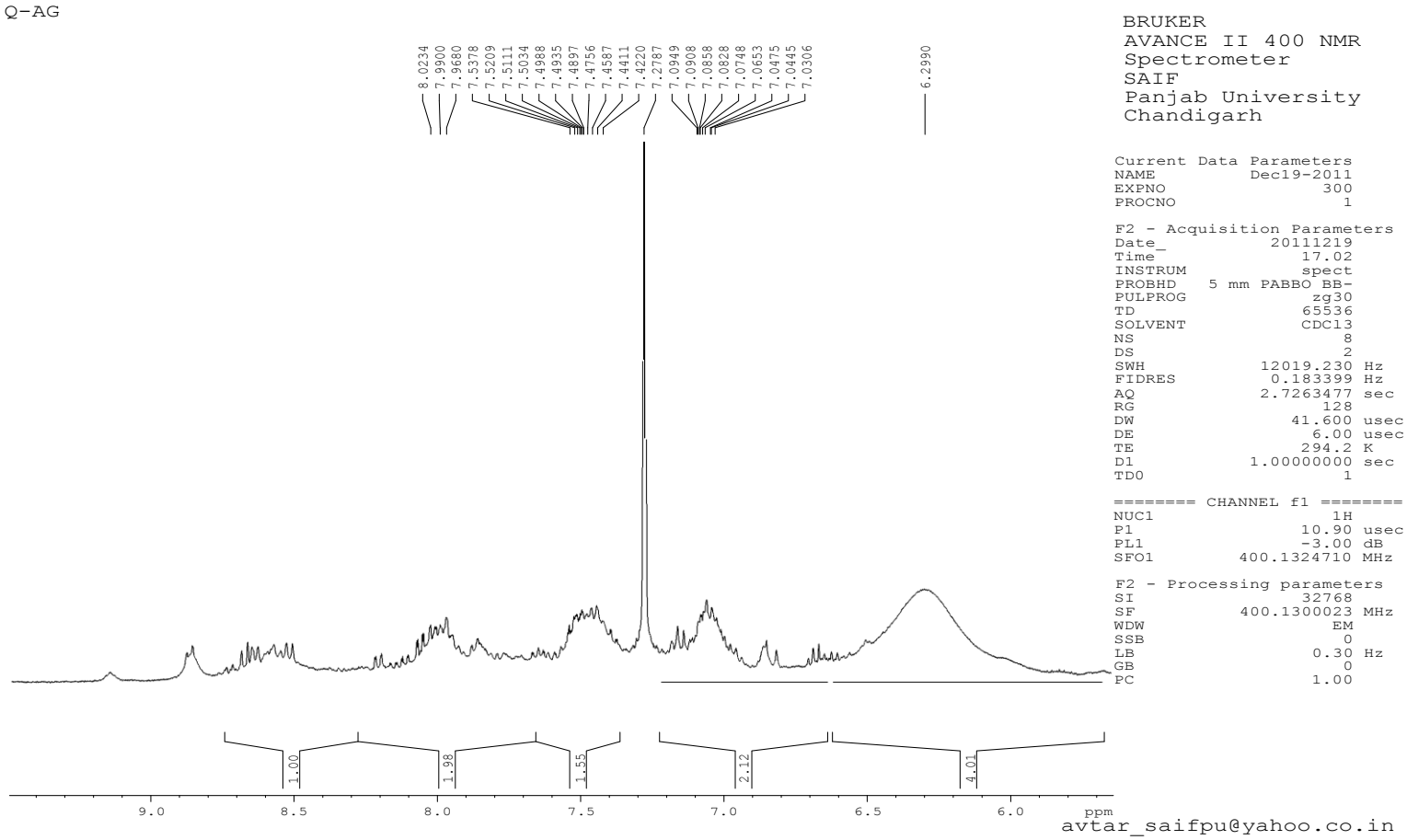


Figure 26: NMR Spectra of QAG

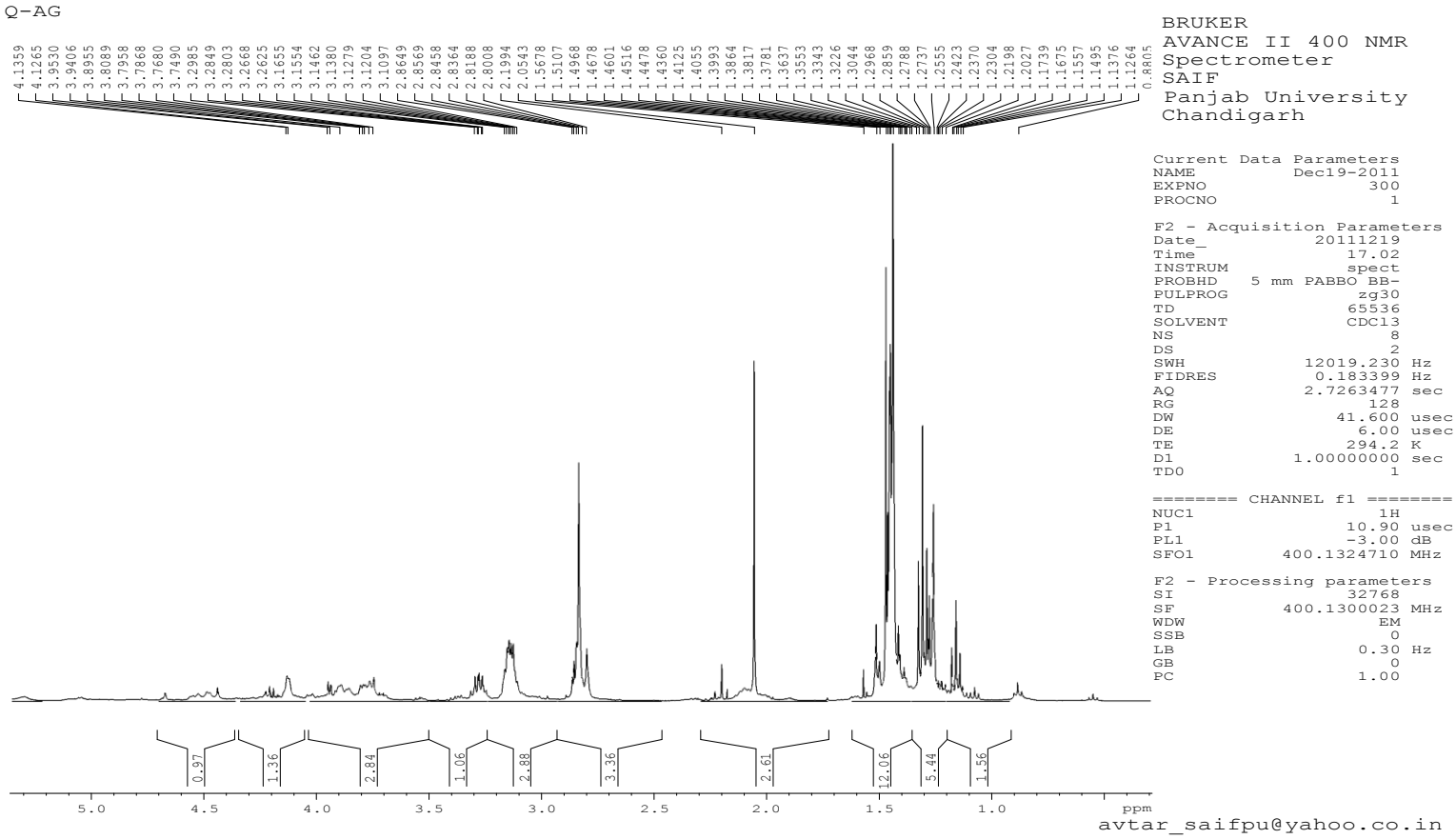
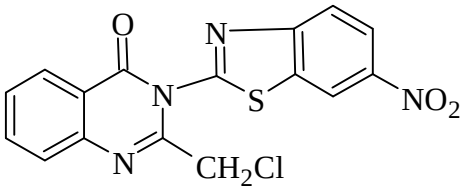


Figure 27: NMR Spectra of QAG

Interpretation of ^{1}H NMR spectra of QBEN



The δ values with reference to the nature of protons are given below**.**

Table-18

S. No	Values in ppm	Nature of protons
1	8.0 – 8.1	Protons of aromatic ring in benzthiazole
2	7.4 – 7.7	Protons of aromatic ring in quinazolone
3	4.0	Protons in chloromethyl

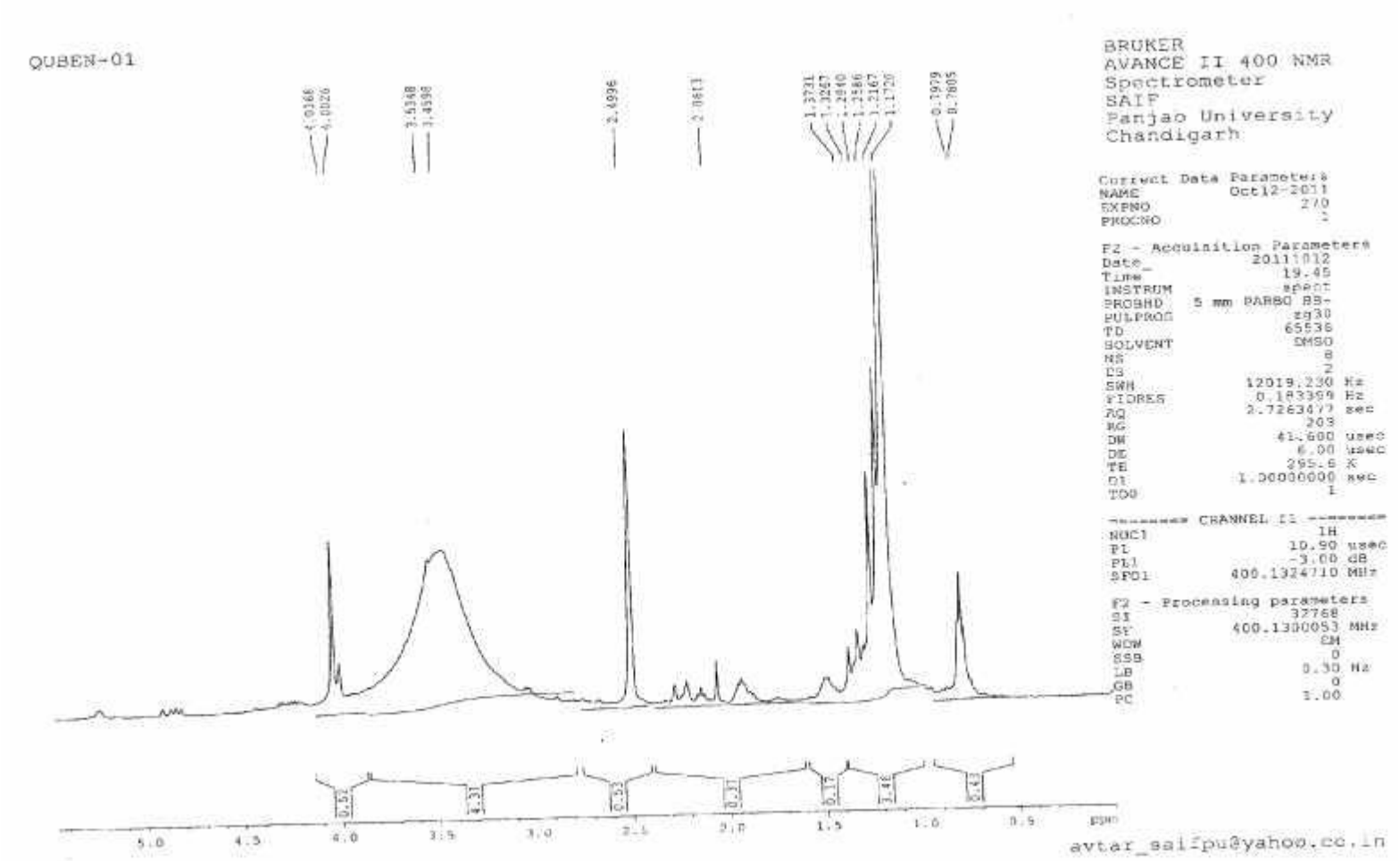


Figure 28: NMR Spectra of QBEN

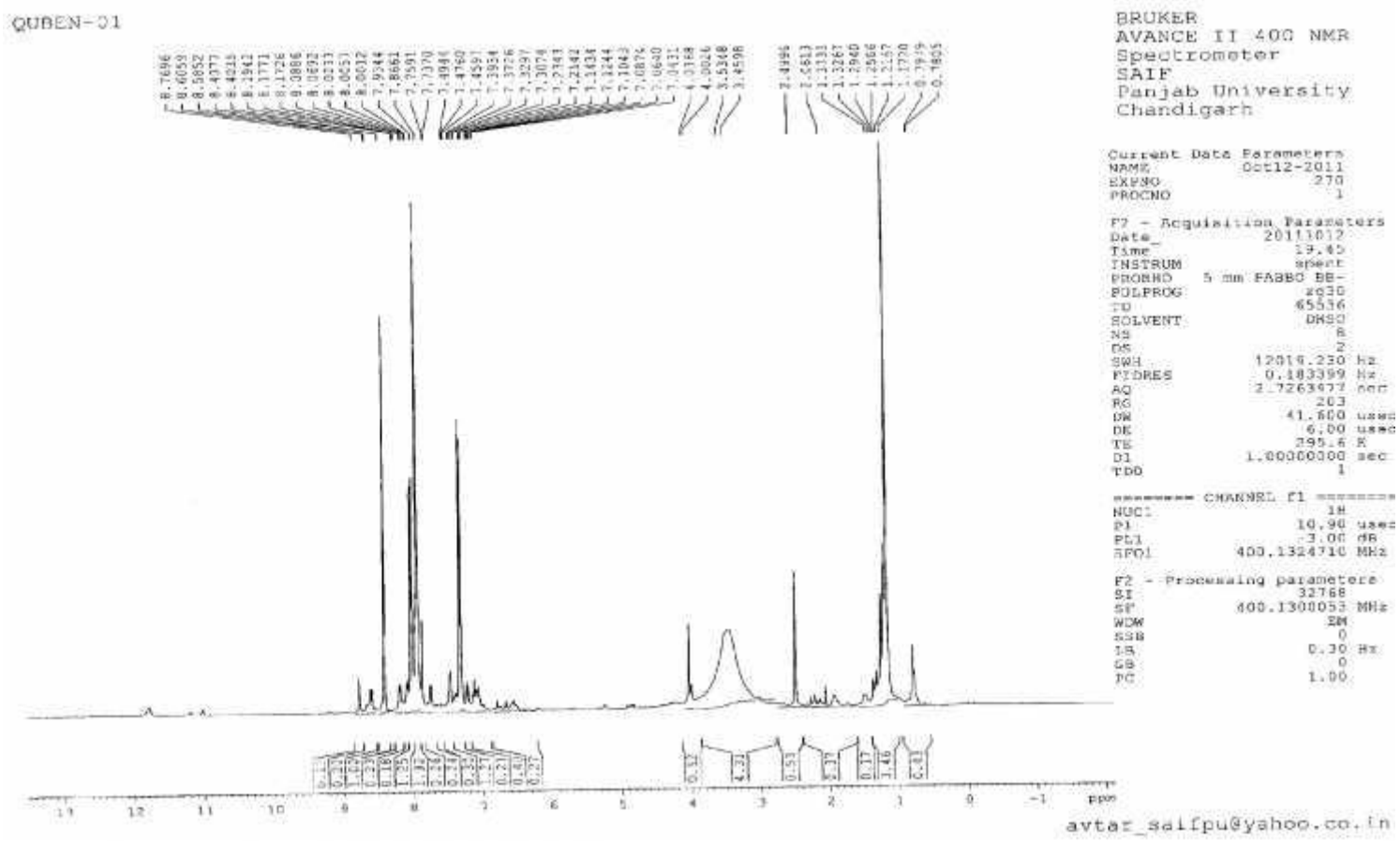


Figure 29: NMR Spectra of QBEN

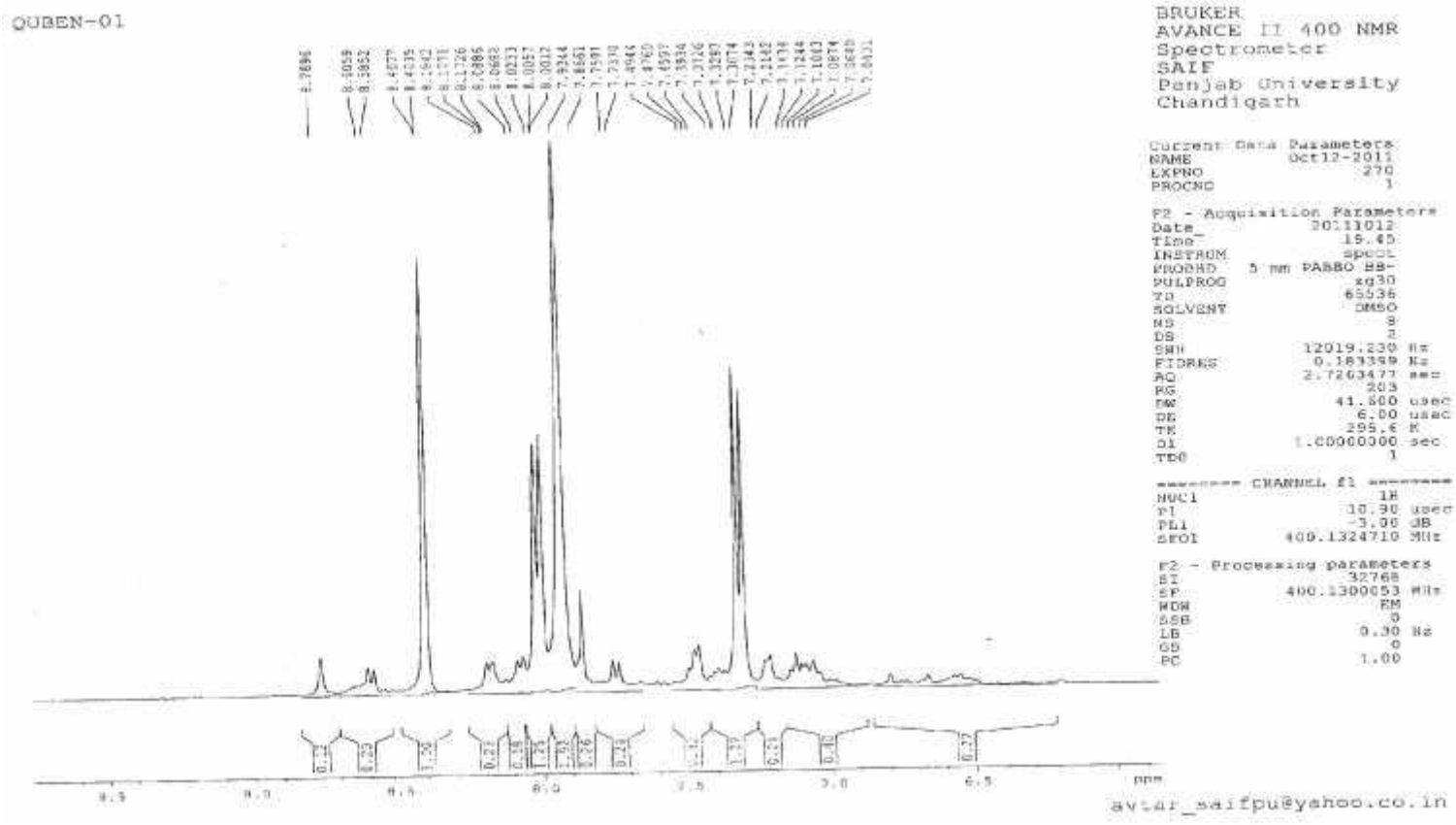
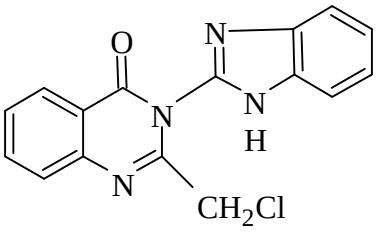


Figure 30: NMR Spectra of QBEN

Interpretation of ^{1}H NMR spectra of QIBM



The δ values with reference to the nature of protons are given below**.**

Table-19

S. No	Values in ppm	Nature of protons
1	7.8	Protons of aromatic ring in quinazolone
2	7.1	Protons of aromatic ring in Benzimidazole
3	5.2	Protons in chloromethetheyl
4	5.0	Protons of secondary amine

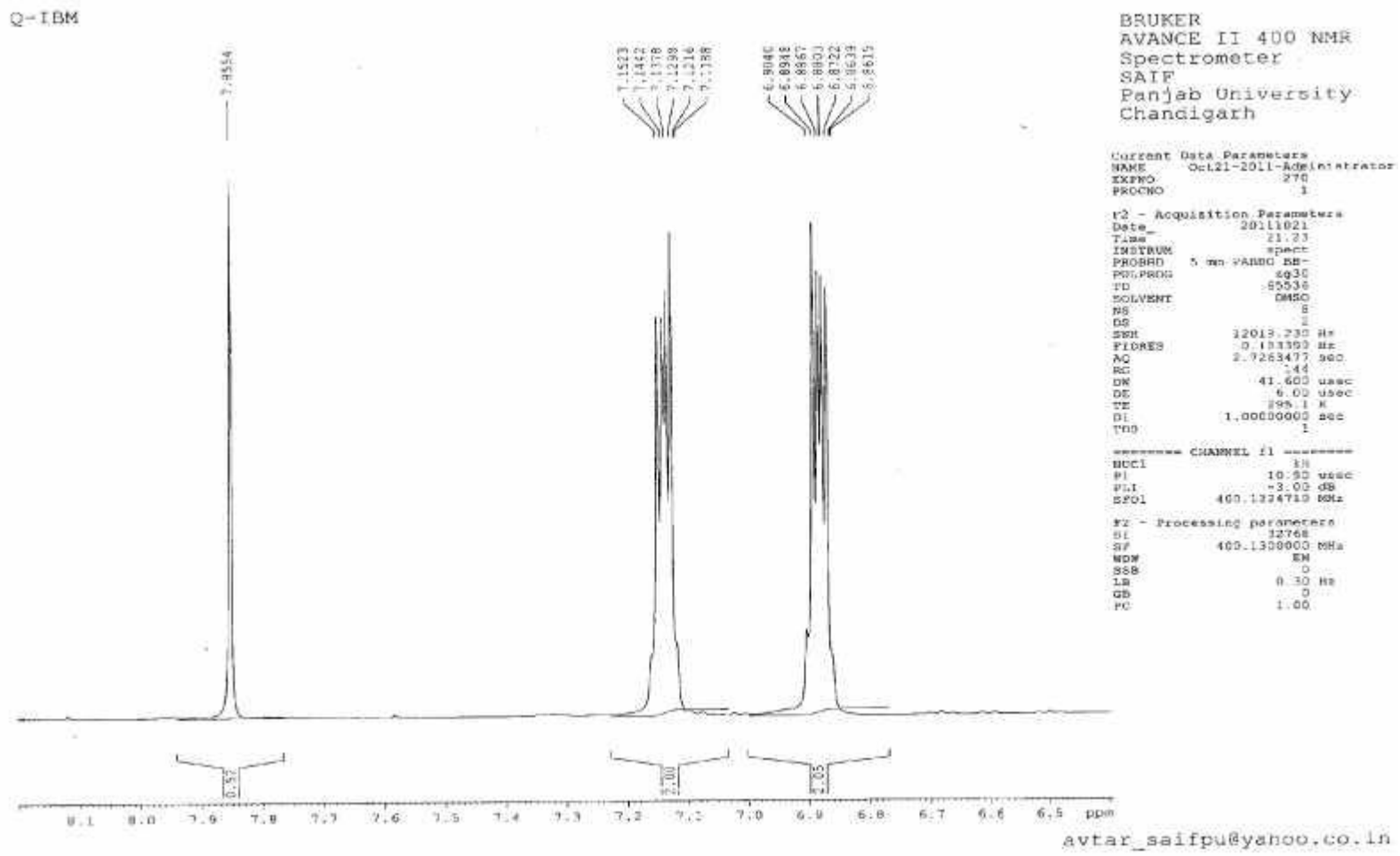


Figure 31: NMR Spectra of QIBM

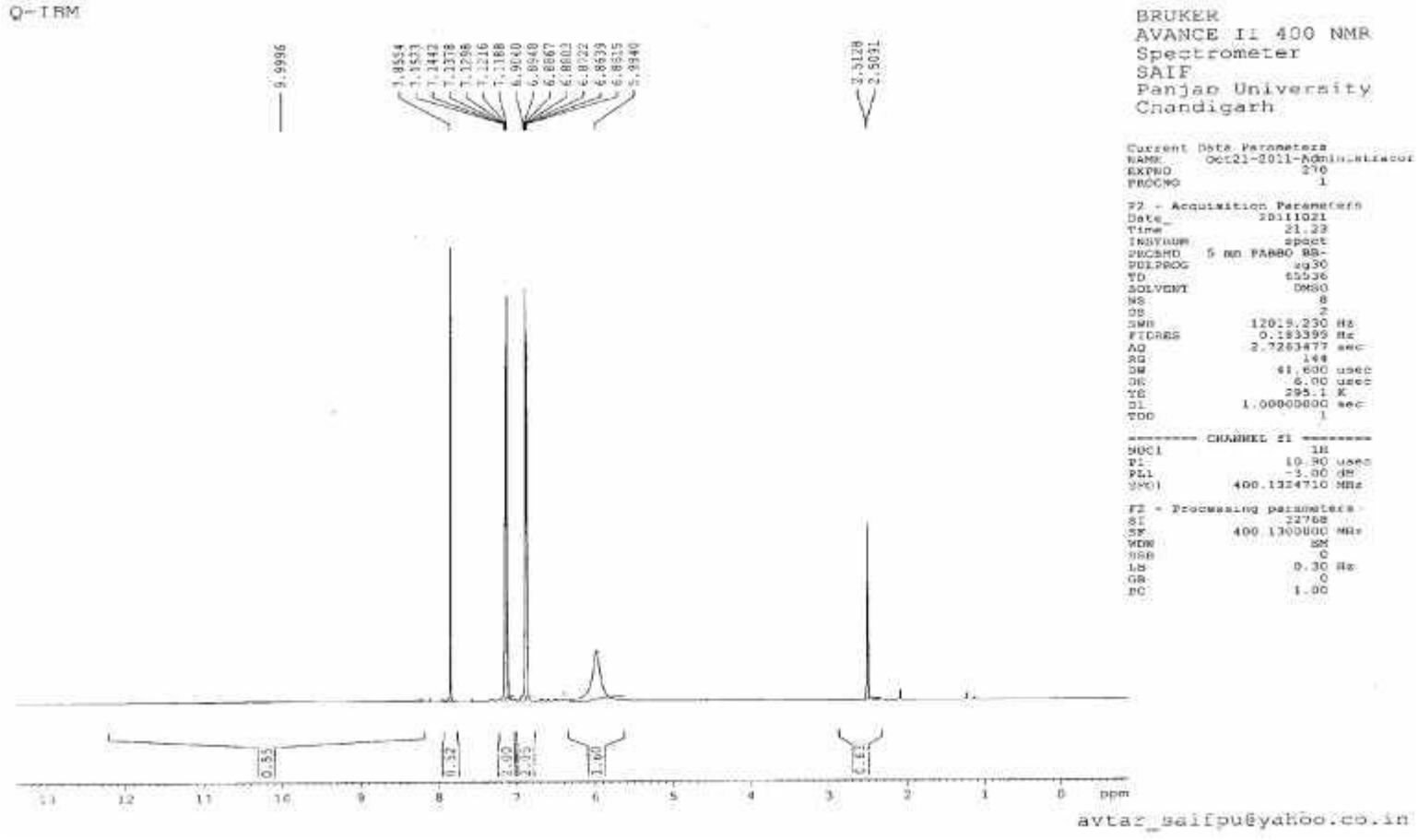
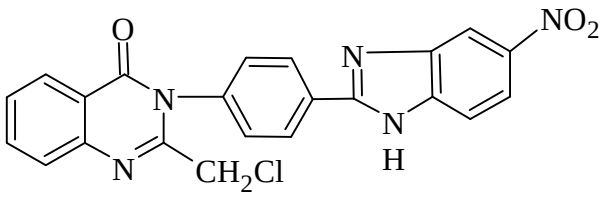


Figure 32: NMR Spectra of QIBM

Interpretation of ^{1}H NMR spectra of QNIBM



The δ values with reference to the nature of protons are given below**.**

Table-20

S. No	Values in ppm	Nature of protons
1	8.1	Protons of aromatic ring in quinazolone
2	7.3–7.8	Protons in phenyl group
3	7.0	Protons of aromatic ring in Benzimidazole
4	4.1-4.3	Protons in chlorometheyl
5	5.0	Protons of secondary amine

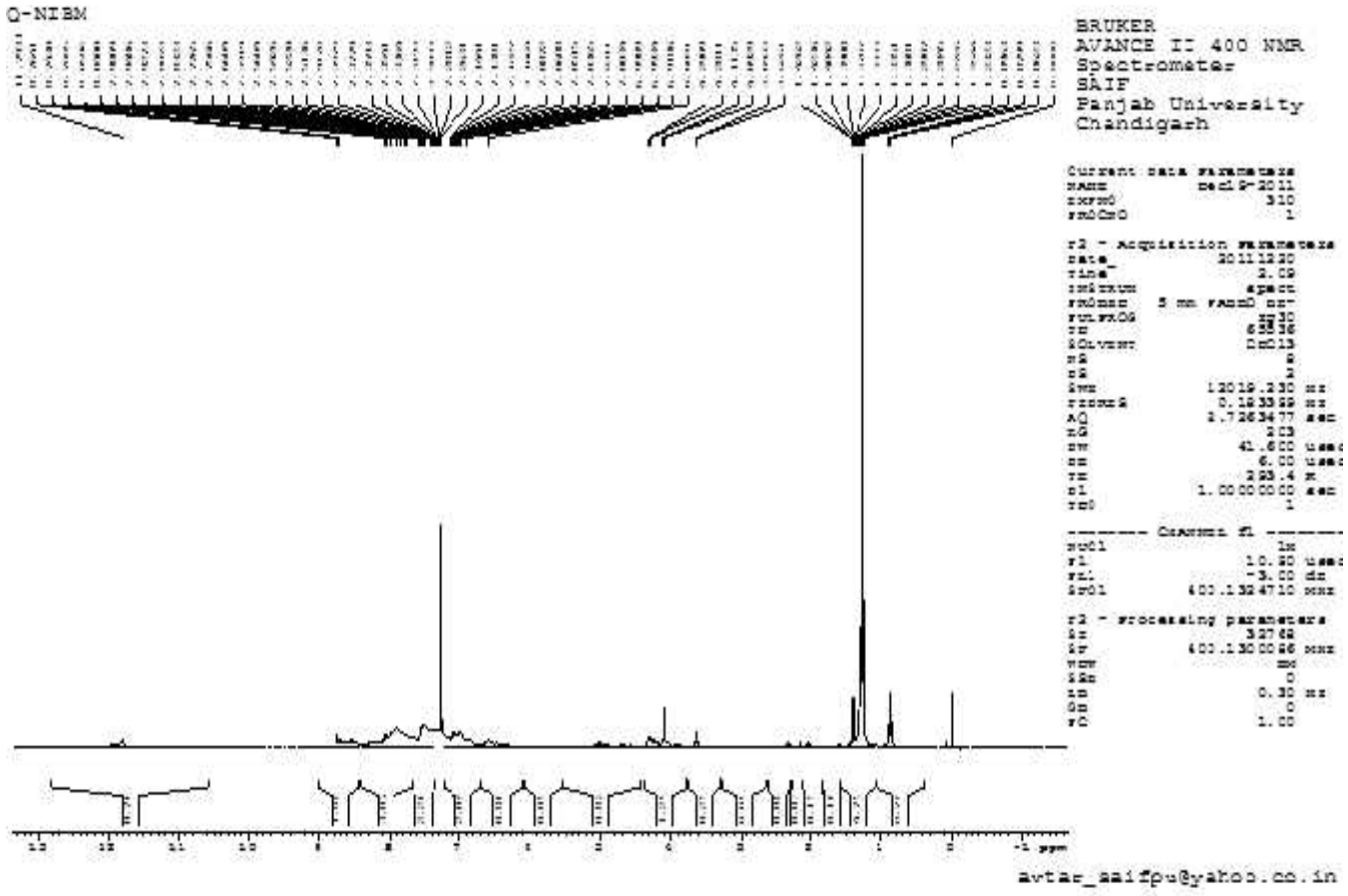


Figure 33: NMR Spectra of QNIBM

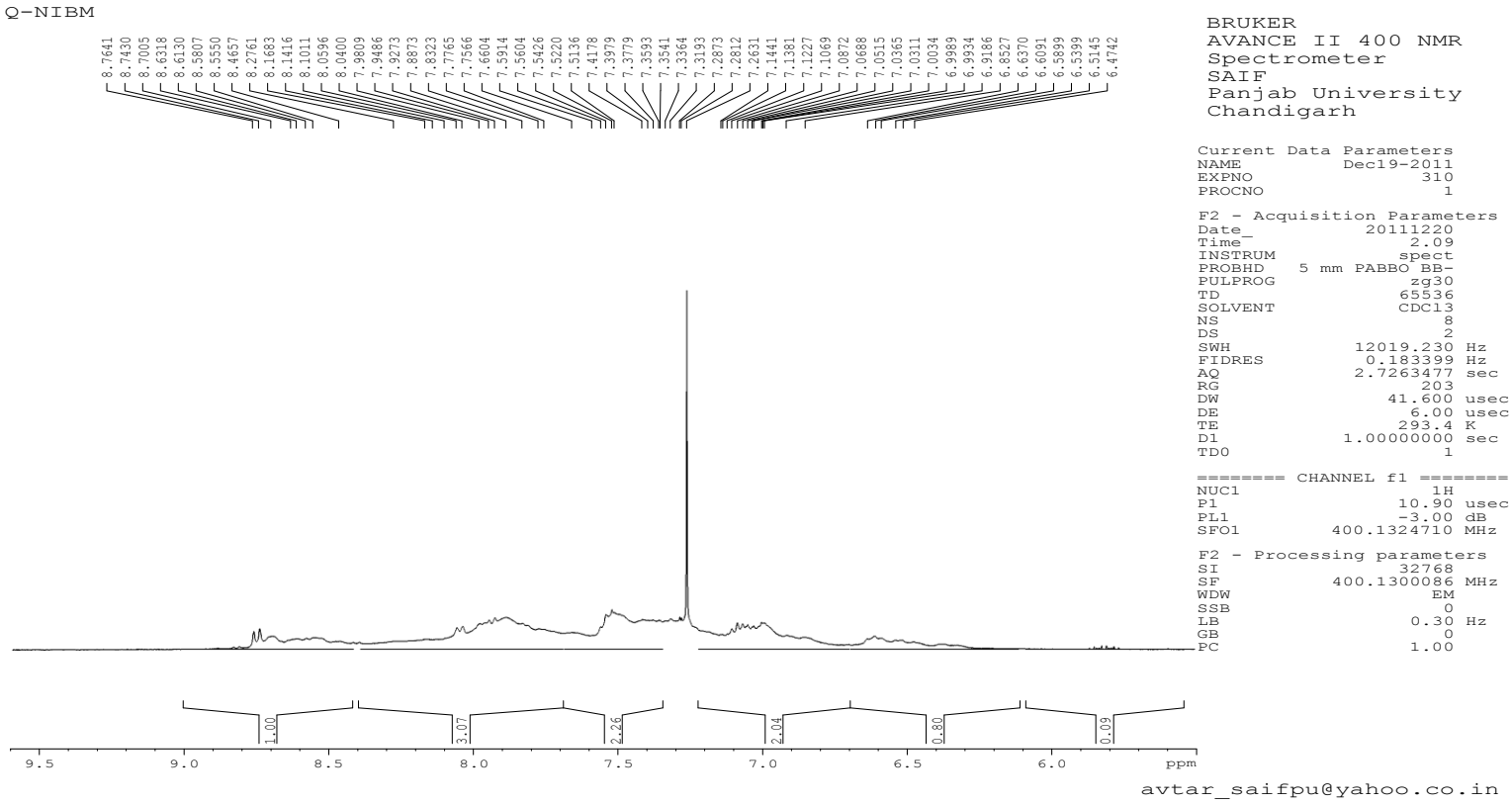


Figure 34: NMR Spectra of QNIBM

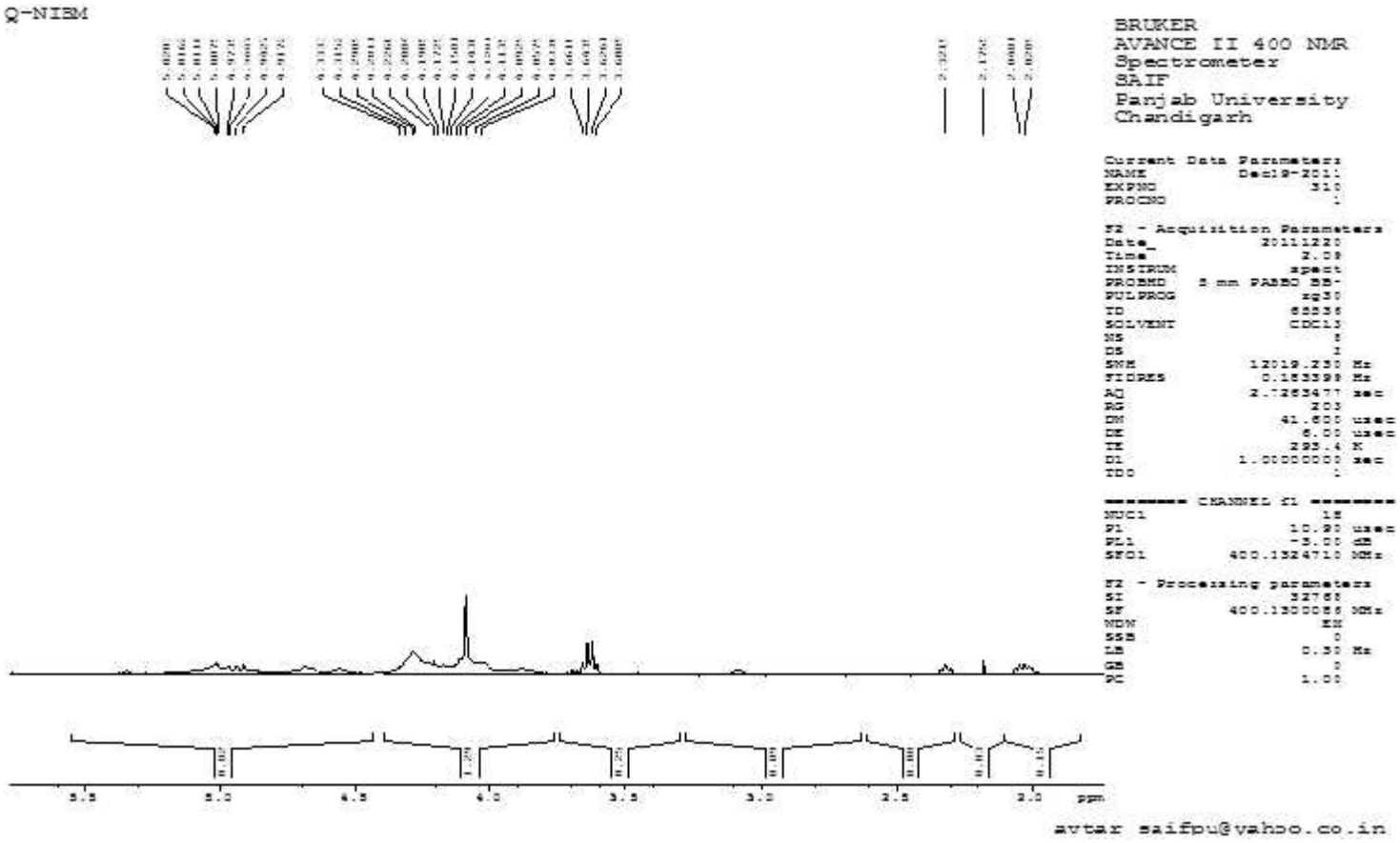


Figure 35: NMR Spectra of QNIBM

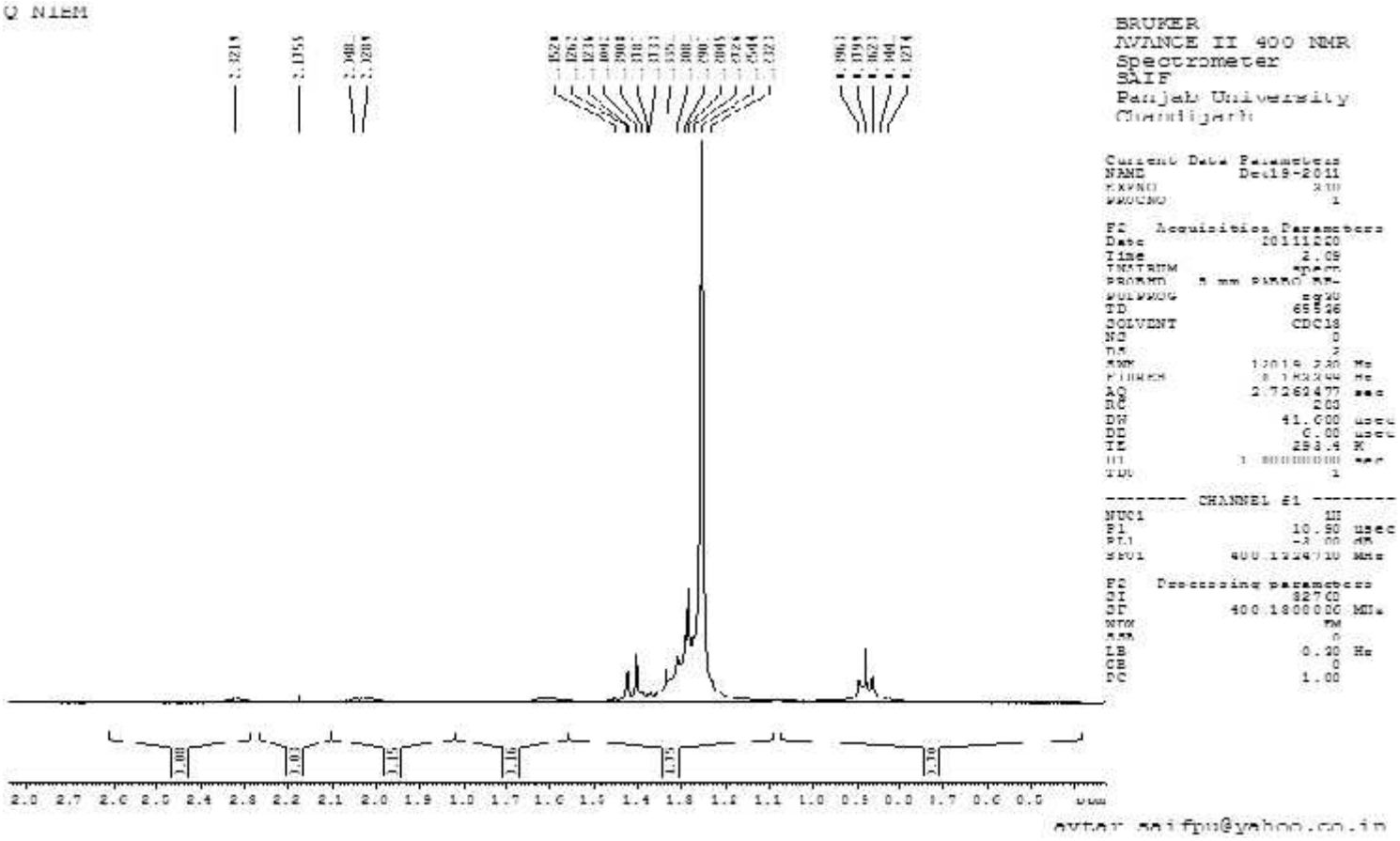
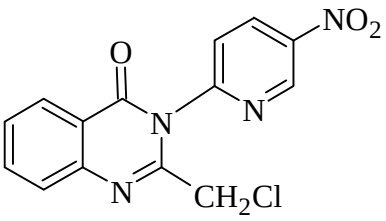


Figure 36: NMR Spectra of QNIBM

Interpretation of ^{1}H NMR spectra of QPIN



The δ values with reference to the nature of protons are given below**.**

Table-21

S. No	Values in ppm	Nature of protons
1	8.5	Protons of aromatic ring in pyridine
2	7.7-8.1	Protons of aromatic ring in quinazolone
3	4.1-4.4	Protons in chloromethyl

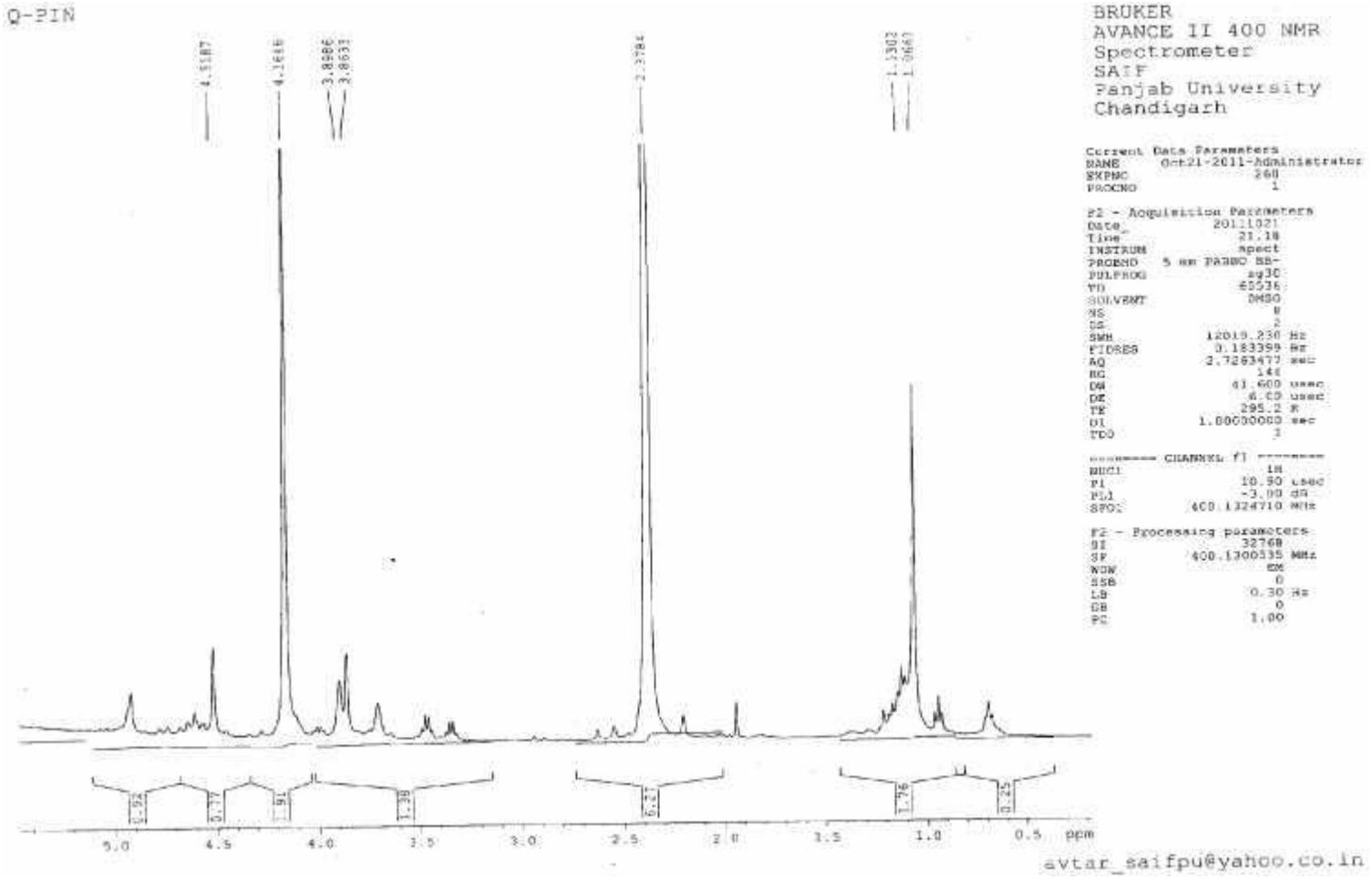


Figure 37: NMR Spectra of QPIN

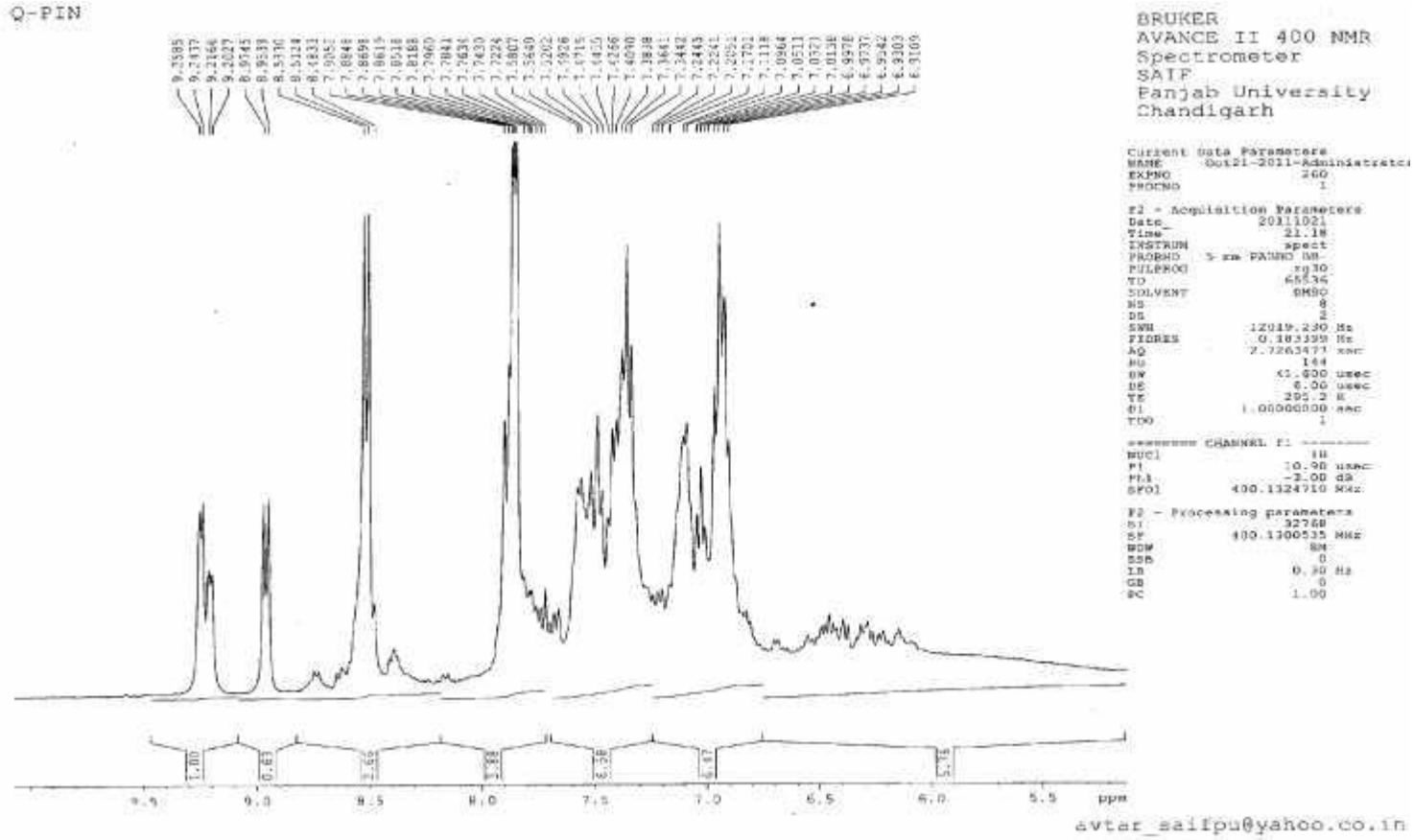
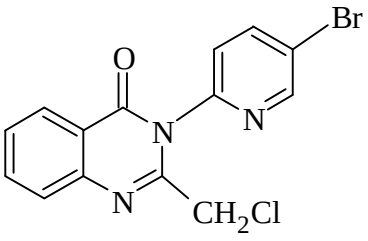


Figure 38: NMR Spectra of QPIN

Interpretation of ^{1}H NMR spectra of QBrP



The δ values with reference to the nature of protons are given below**.**

Table-22

S. No	Values in ppm	Nature of protons
1	8.6	Protons of aromatic ring in pyridine
2	8.1	Protons of aromatic ring in quinazolone
3	4.1-4.4	Protons in chloromethyl

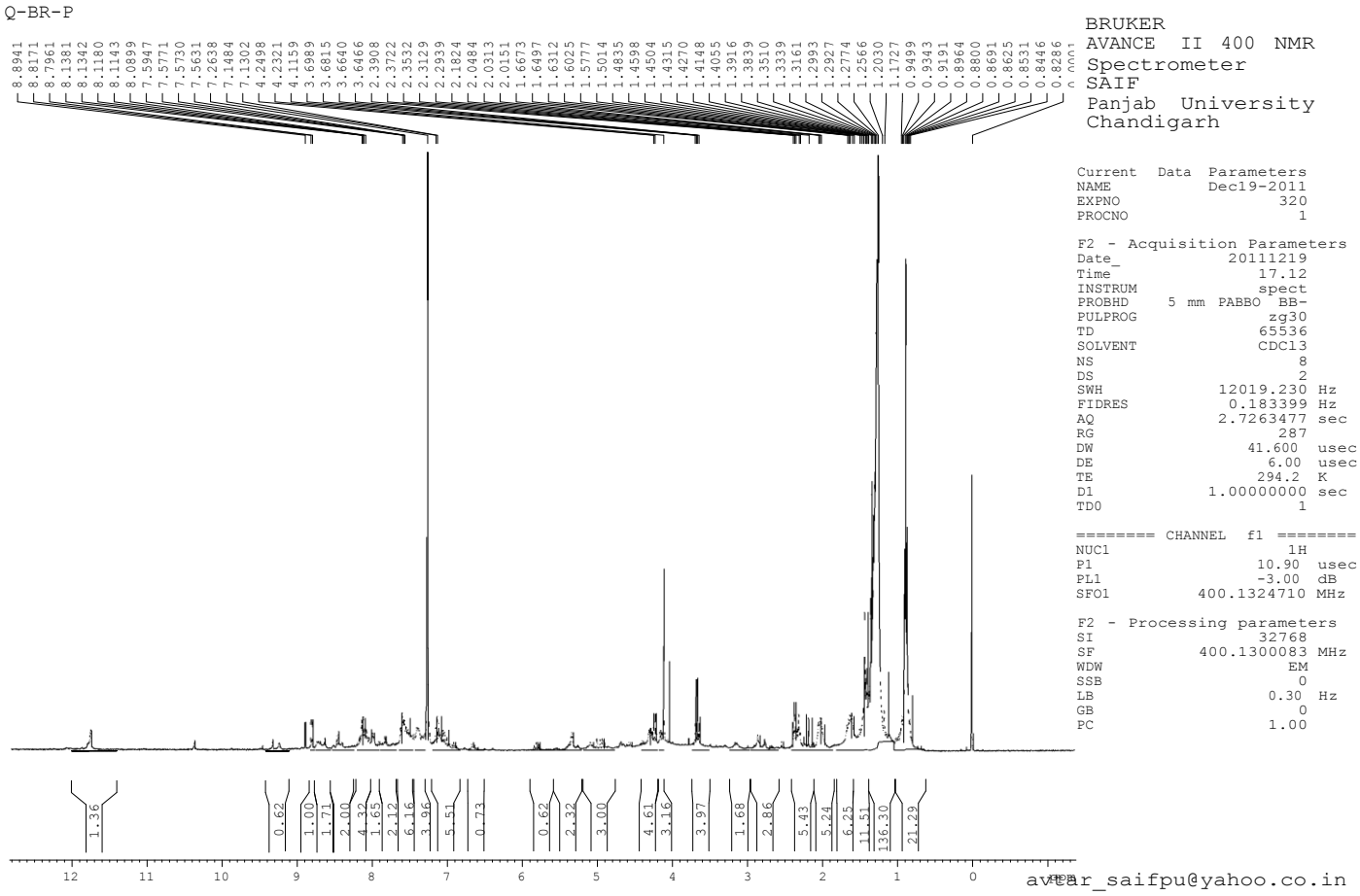


Figure 39: NMR Spectra of QPIN

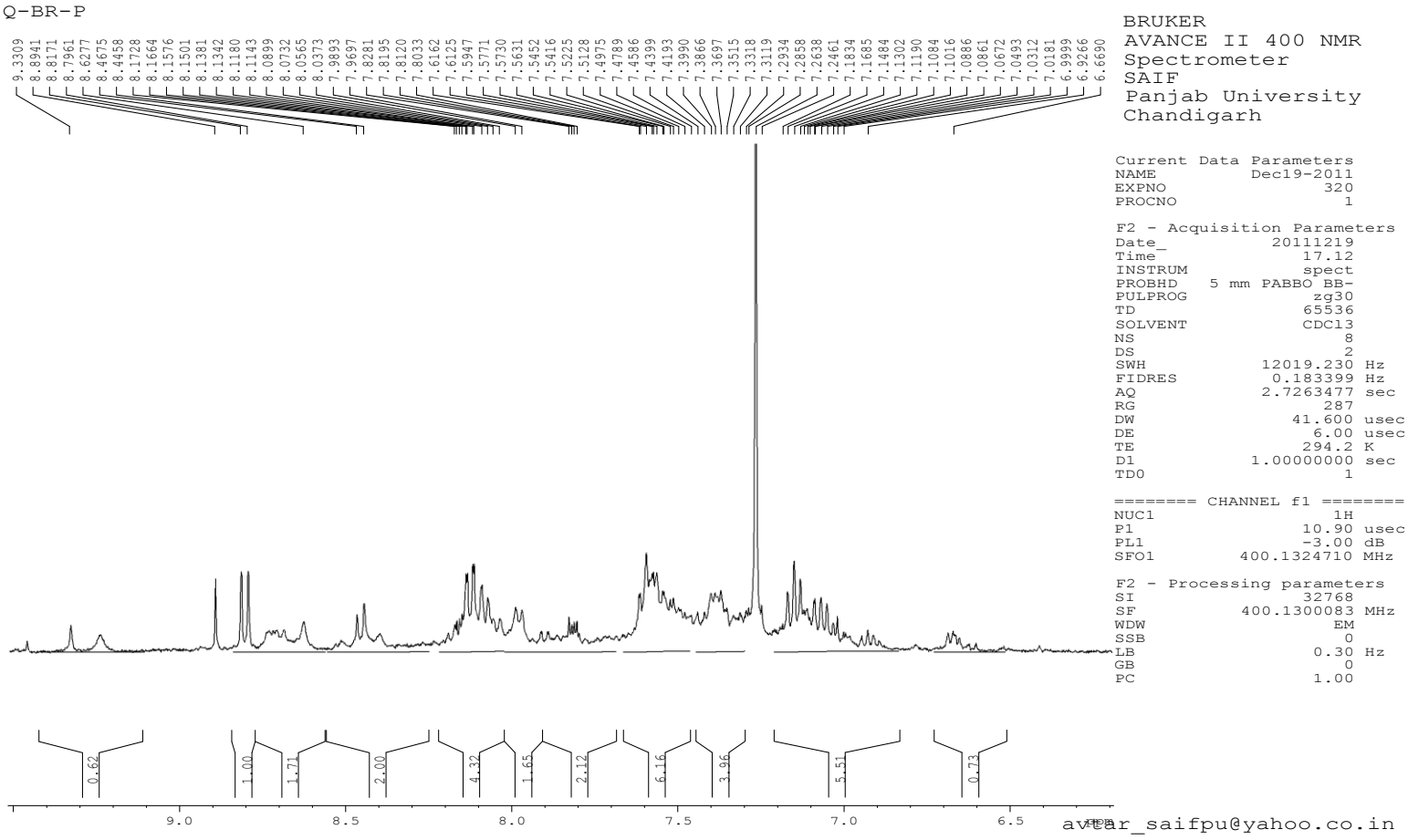


Figure 40: NMR Spectra of QBrP

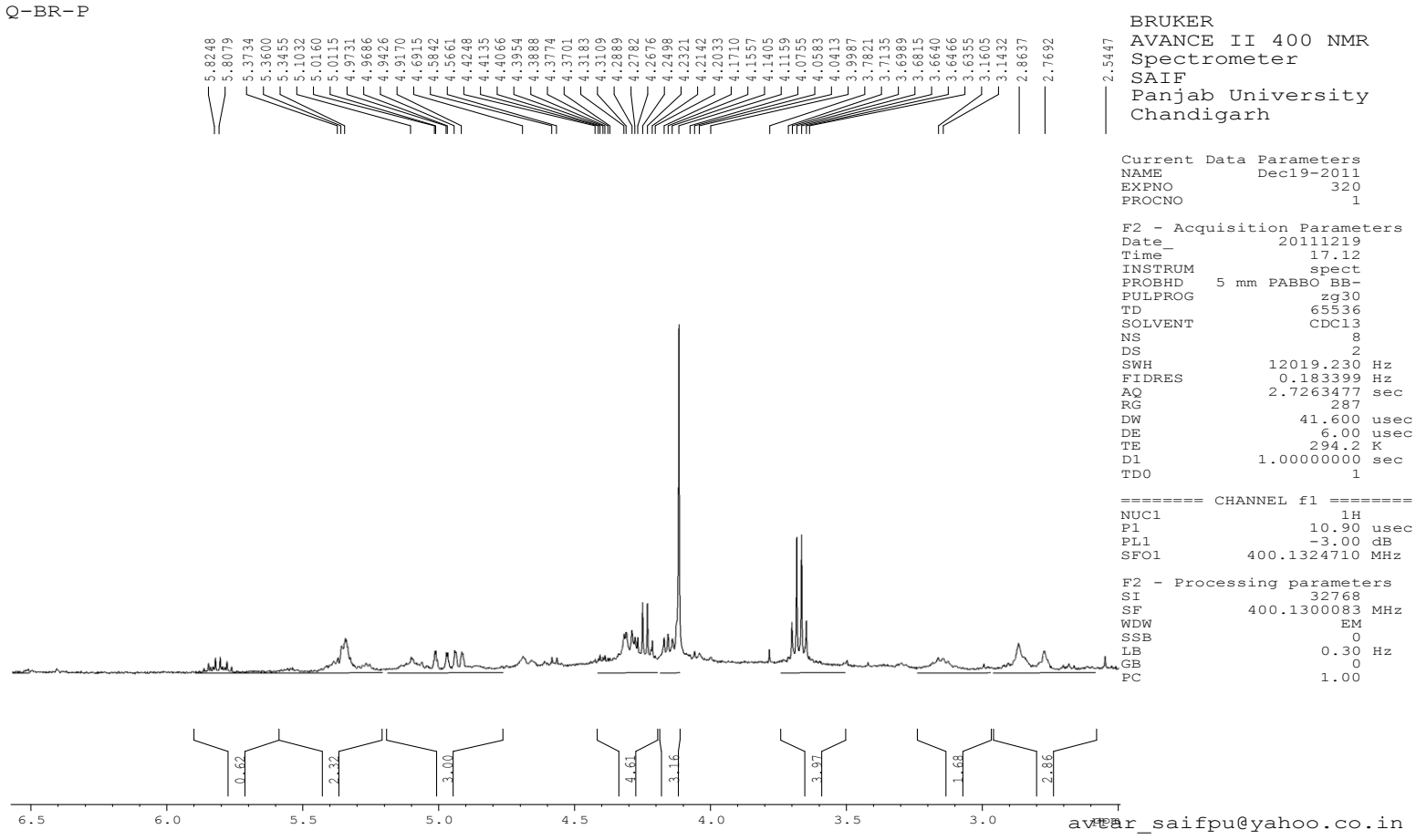


Figure 41: NMR Spectra of QBrP

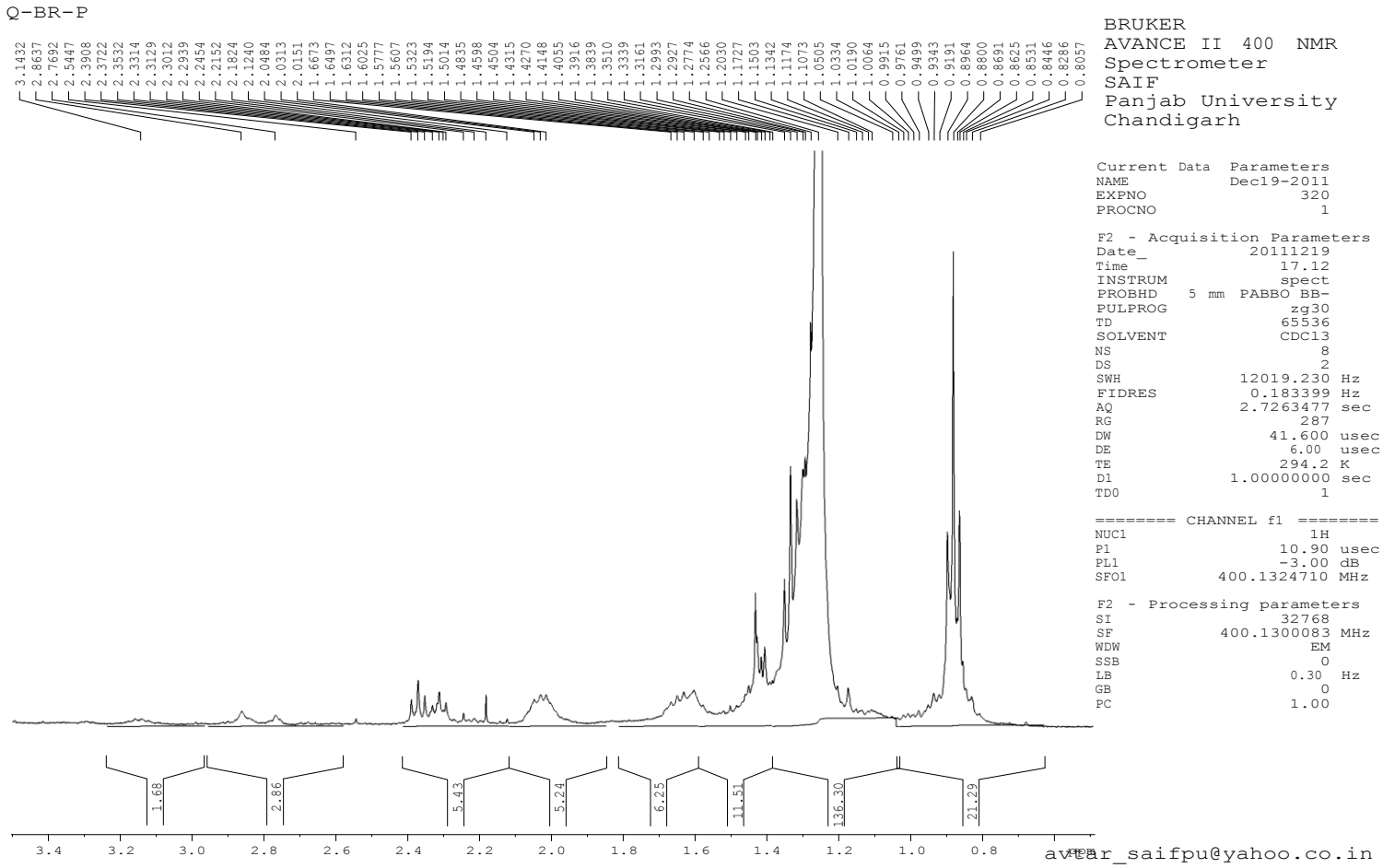
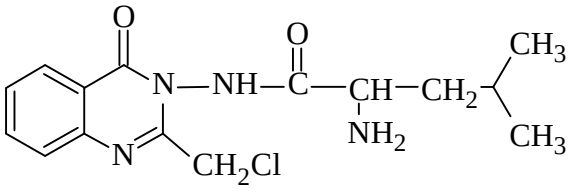


Figure 42: NMR Spectra of QBrP

iii) Mass Spectral Analysis

Mass spectrum of the sample was recorded in JEOL GCmate by Electron impact method as ionization mode.

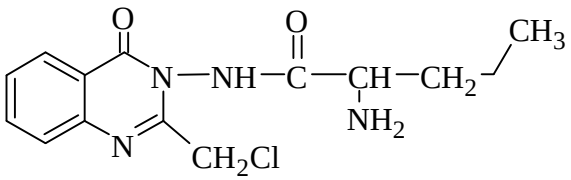
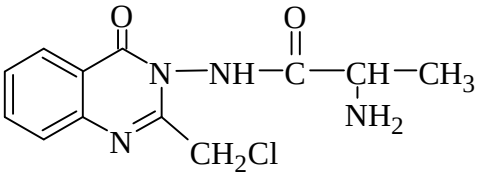
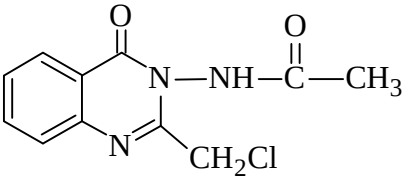
Interpretation of Mass spectra of QAL



Molecular Weight: 322.78 Molecular Ion Peak: 322.78

The possible fragments of the molecule with the relevant to its m/z values are:

Table-23

S. No	m/z	Fragments
1	309.52	
2	281.70	
3	252.61	

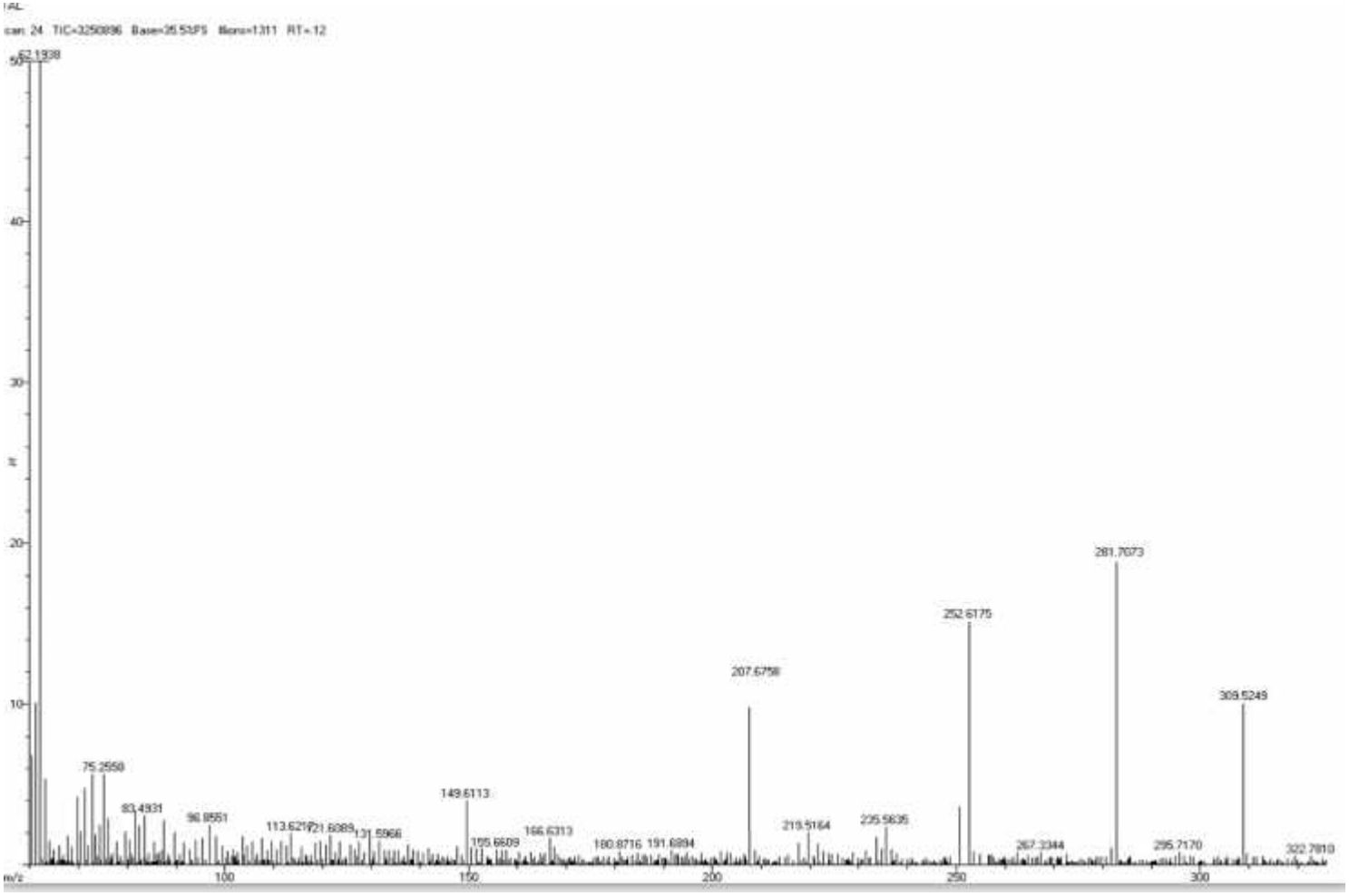
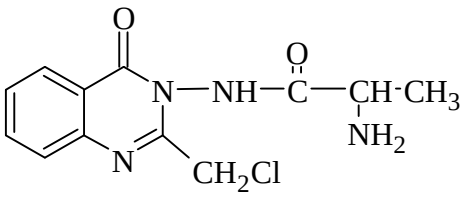


Figure 43: Mass Spectra of QAL

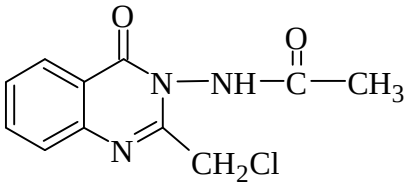
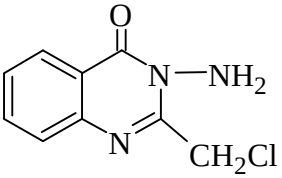
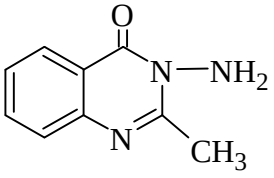
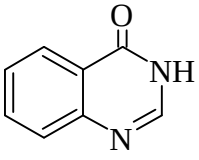
Interpretation of Mass spectra of QAA



Molecular Weight: 280.71 Molecular Ion Peak: 280.71

The possible fragments of the molecule with the relevant to its m/z values are:

Table-24

S. No	m/z	Fragments
1	251.62	
2	218.62	
3	184.71	
4	148	

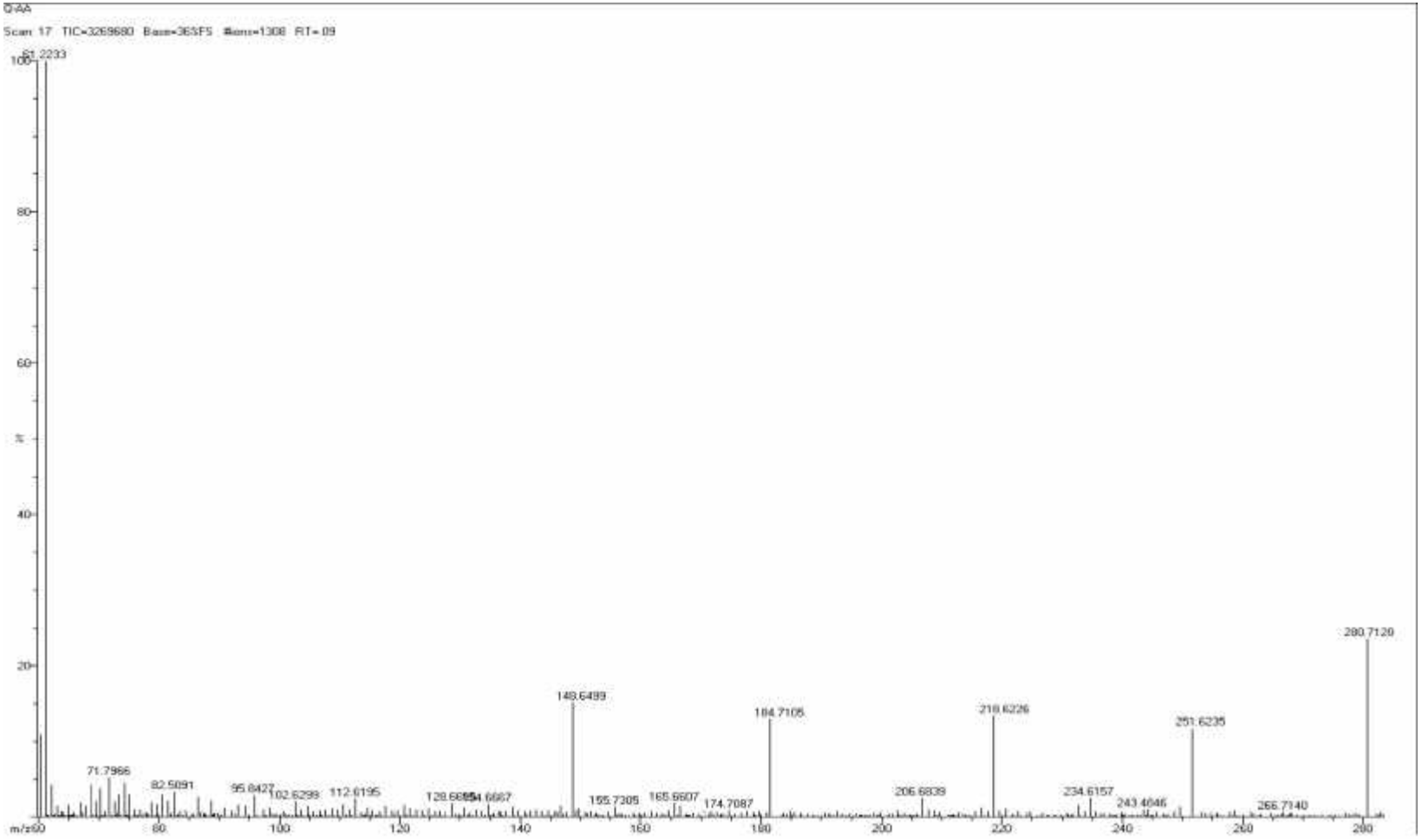
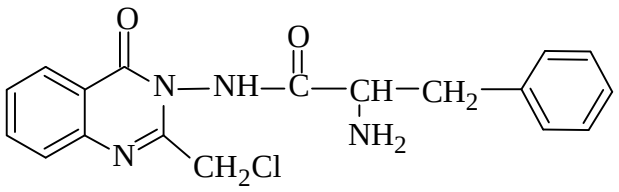


Figure 44: Mass Spectra of QAA

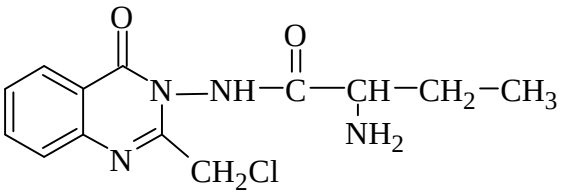
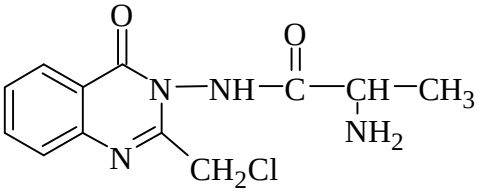
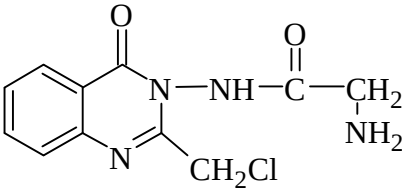
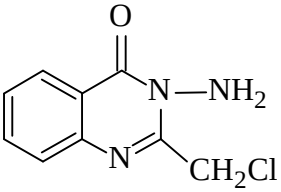
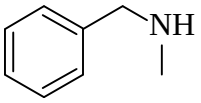
Interpretation of Mass spectra of QAPA



Molecular Weight: 356.80 Molecular Ion Peak: 356.80

The possible fragments of the molecule with the relevant to its m/z values are:

Table-25

S. No	m/z	Fragments
1	296.73	
2	282.72	
3	253.16	
4	208.69	
5	122.63	

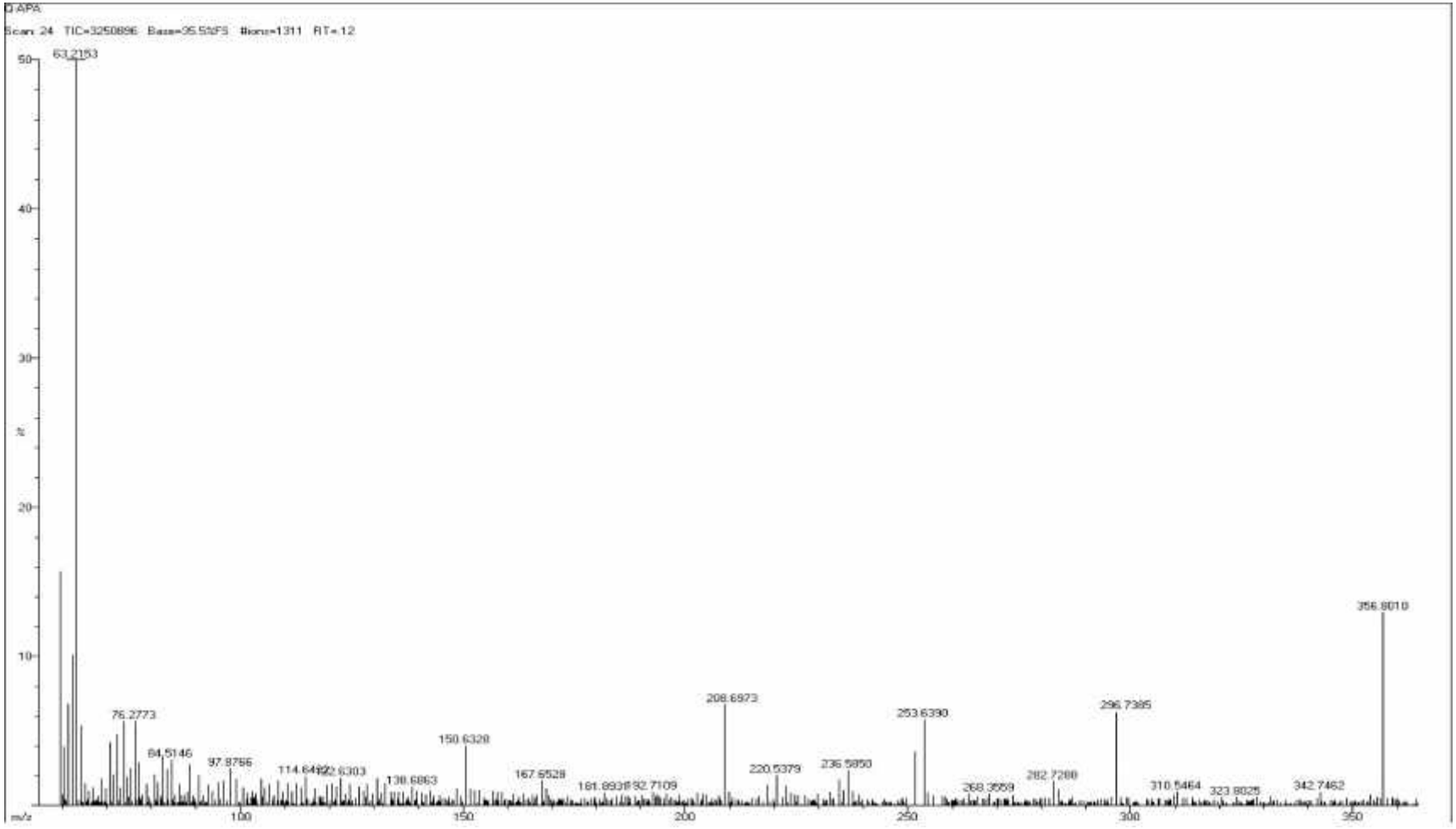
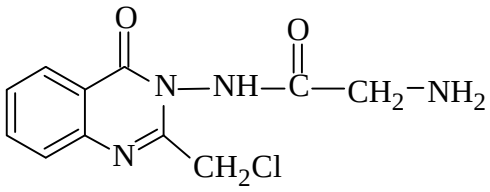


Figure 45: Mass Spectra of QAPA

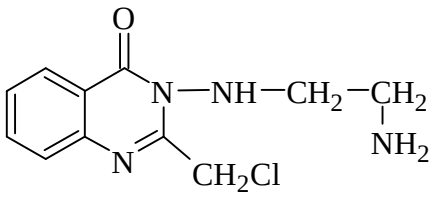
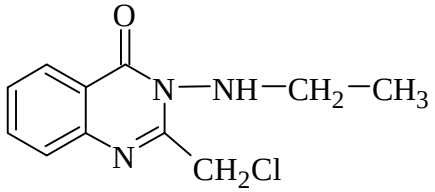
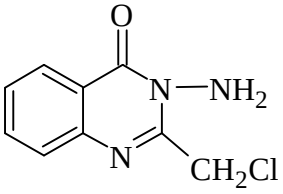
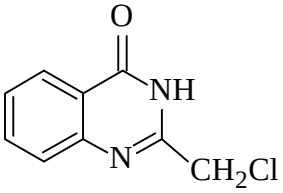
Interpretation of Mass spectra of QAG



Molecular Weight: 266.34 Molecular Ion Peak: 266.68

The possible fragments of the molecule with the relevant to its m/z values are:

Table-26

S. No	m/z	Fragments
1	253.56	
2	236.55	
3	208.62	
4	194.61	

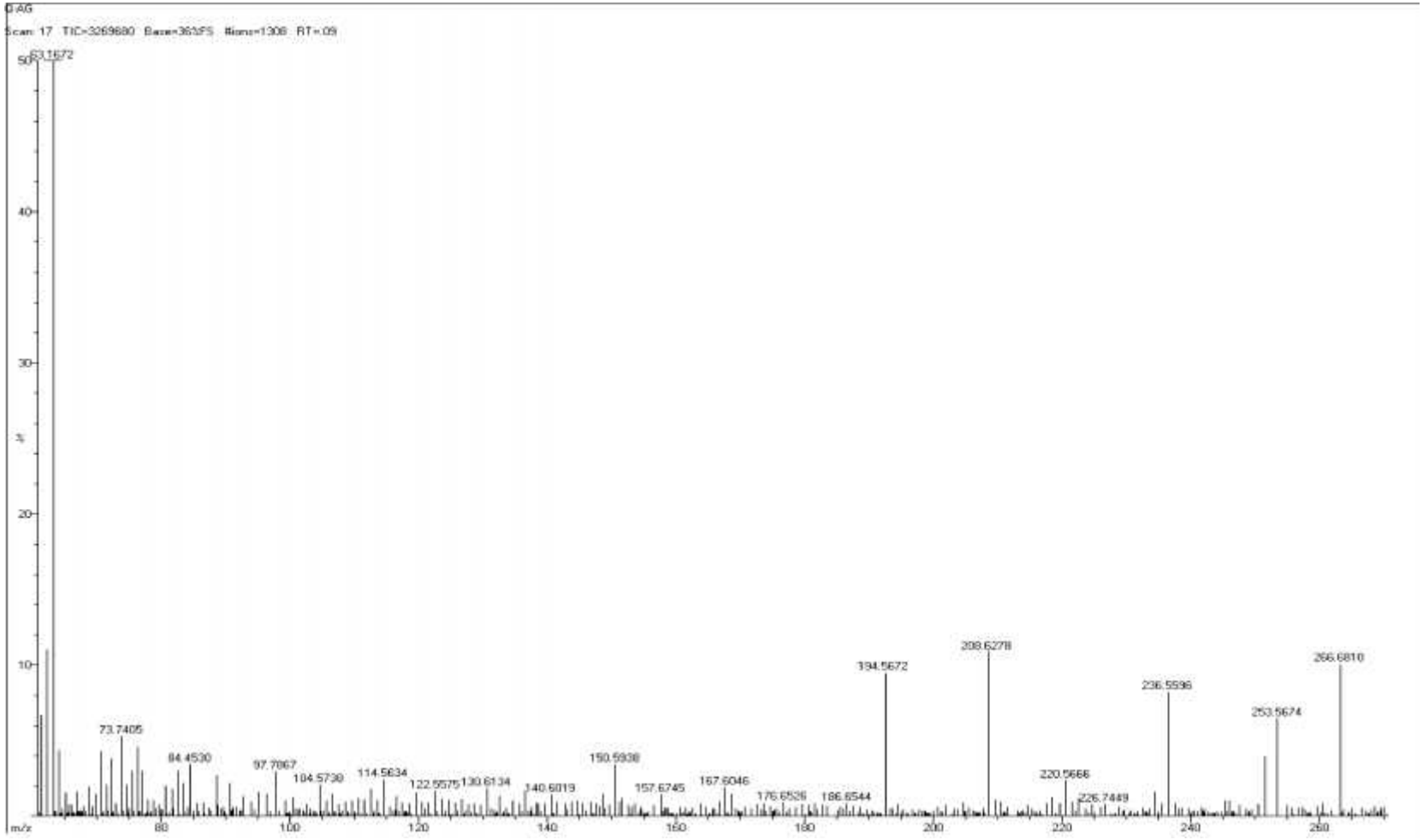
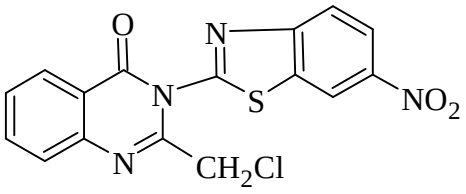


Figure 46: Mass Spectra of QAG

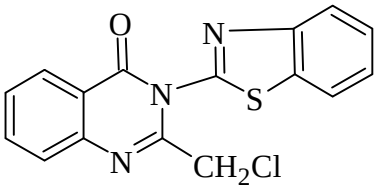
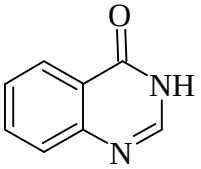
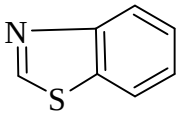
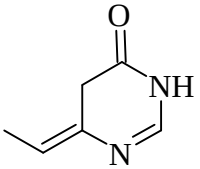
Interpretation of Mass spectra of QBEN



Molecular Weight: 325.87 Molecular Ion Peak: 325.41

The possible fragments of the molecule with the relevant to its m/z values are:

Table-27

S. No	m/z	Fragments
1	275.3	
2	141.4	
3	133.5	
4	119.4	

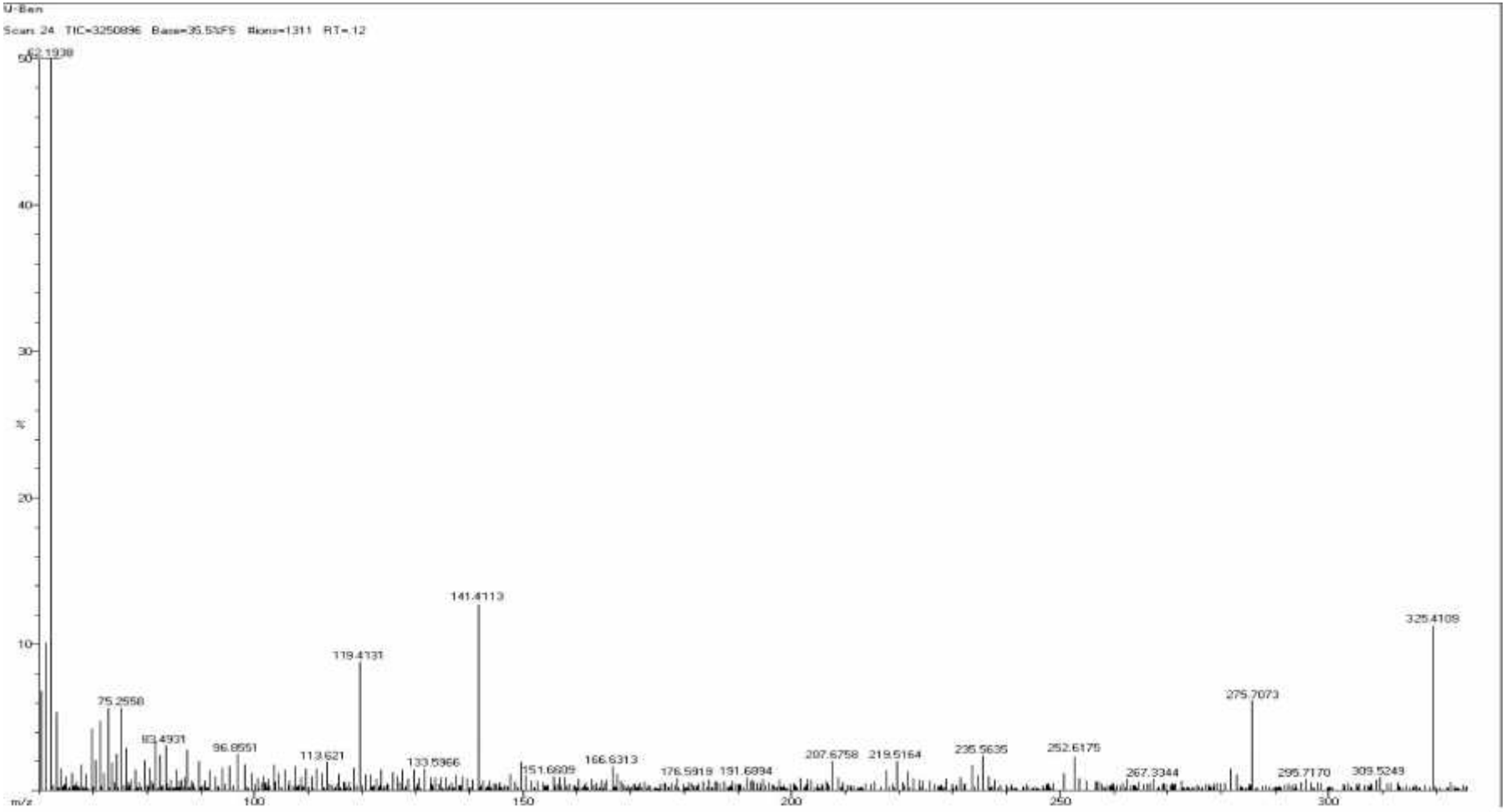
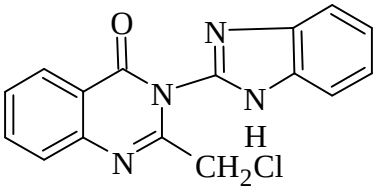


Figure 47: Mass Spectra of QBEN

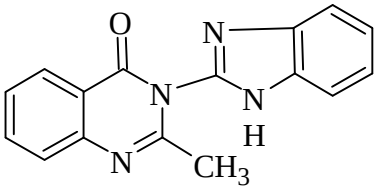
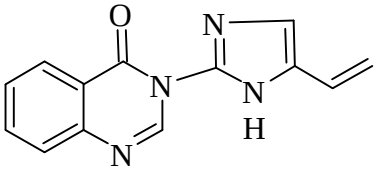
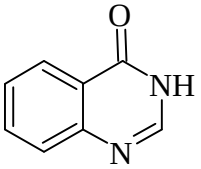
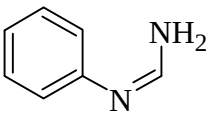
Interpretation of Mass spectra of QIBM



Molecular Weight: 274.27 Molecular Ion Peak: 274.36

The possible fragments of the molecule with the relevant to its m/z values are:

Table-28

S. No	m/z	Fragments
1	261	
2	236	
3	145	
4	117	

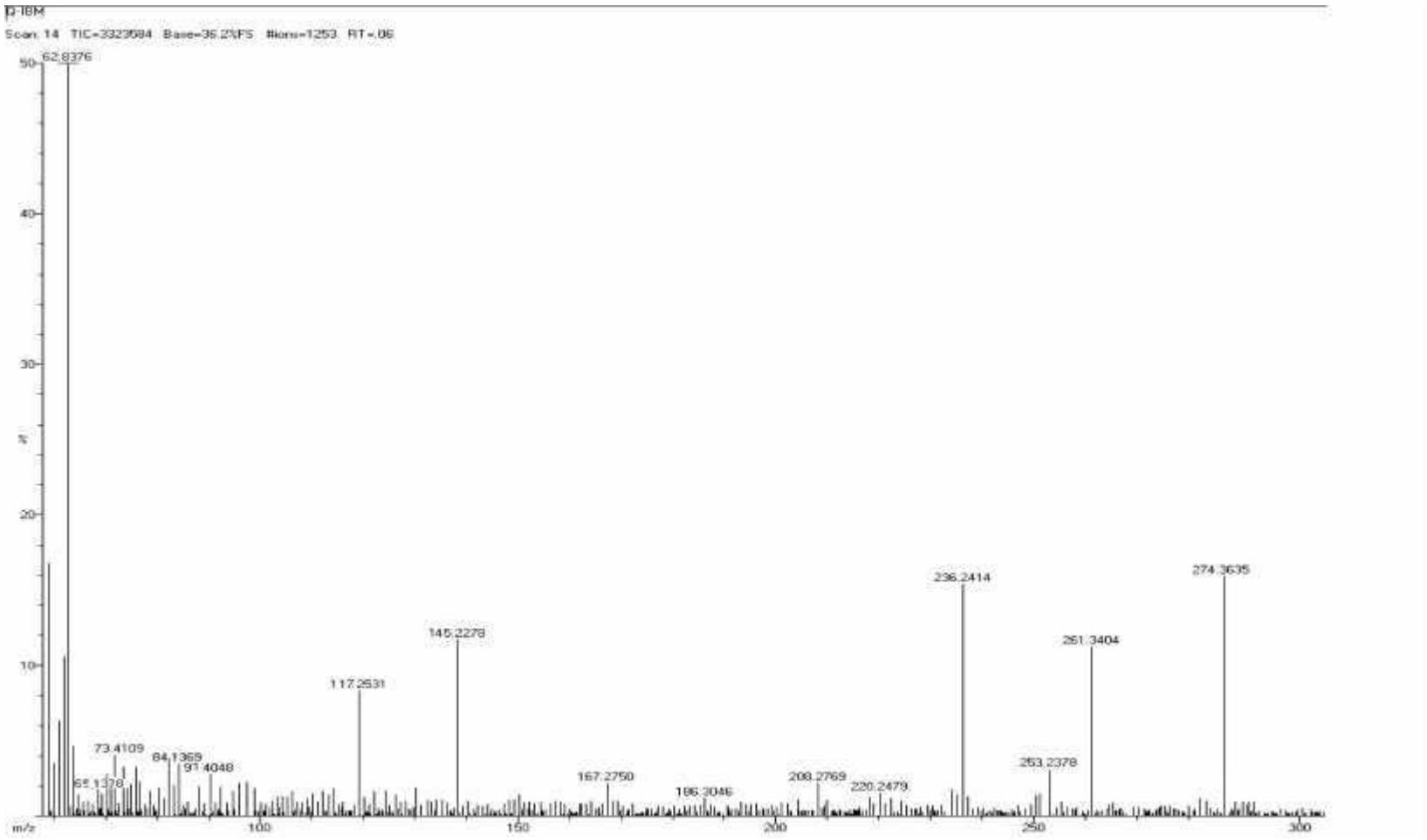
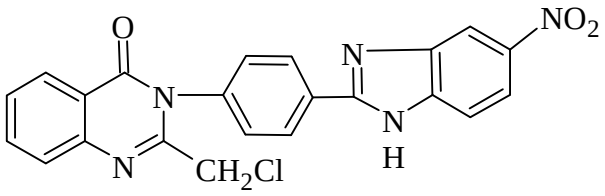


Figure 48: Mass Spectra of QIBM

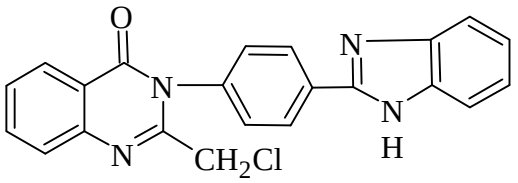
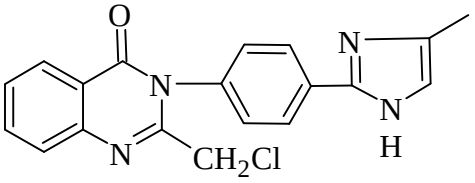
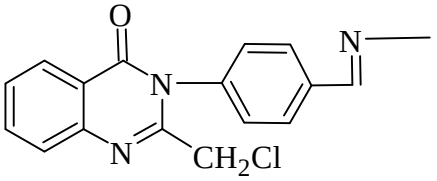
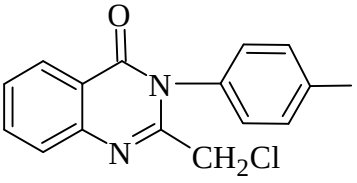
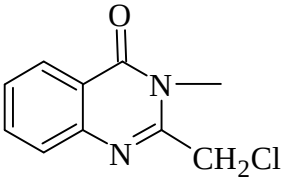
Interpretation of Mass spectra of QNIBM



Molecular Weight: 432.31 Molecular Ion Peak: 432.83

The possible fragments of the molecule with the relevant to its m/z values are:

Table-29

S. No	m/z	Fragments
1	389.5	
2	350.78	
3	312.74	
4	284.72	
5	210.66	

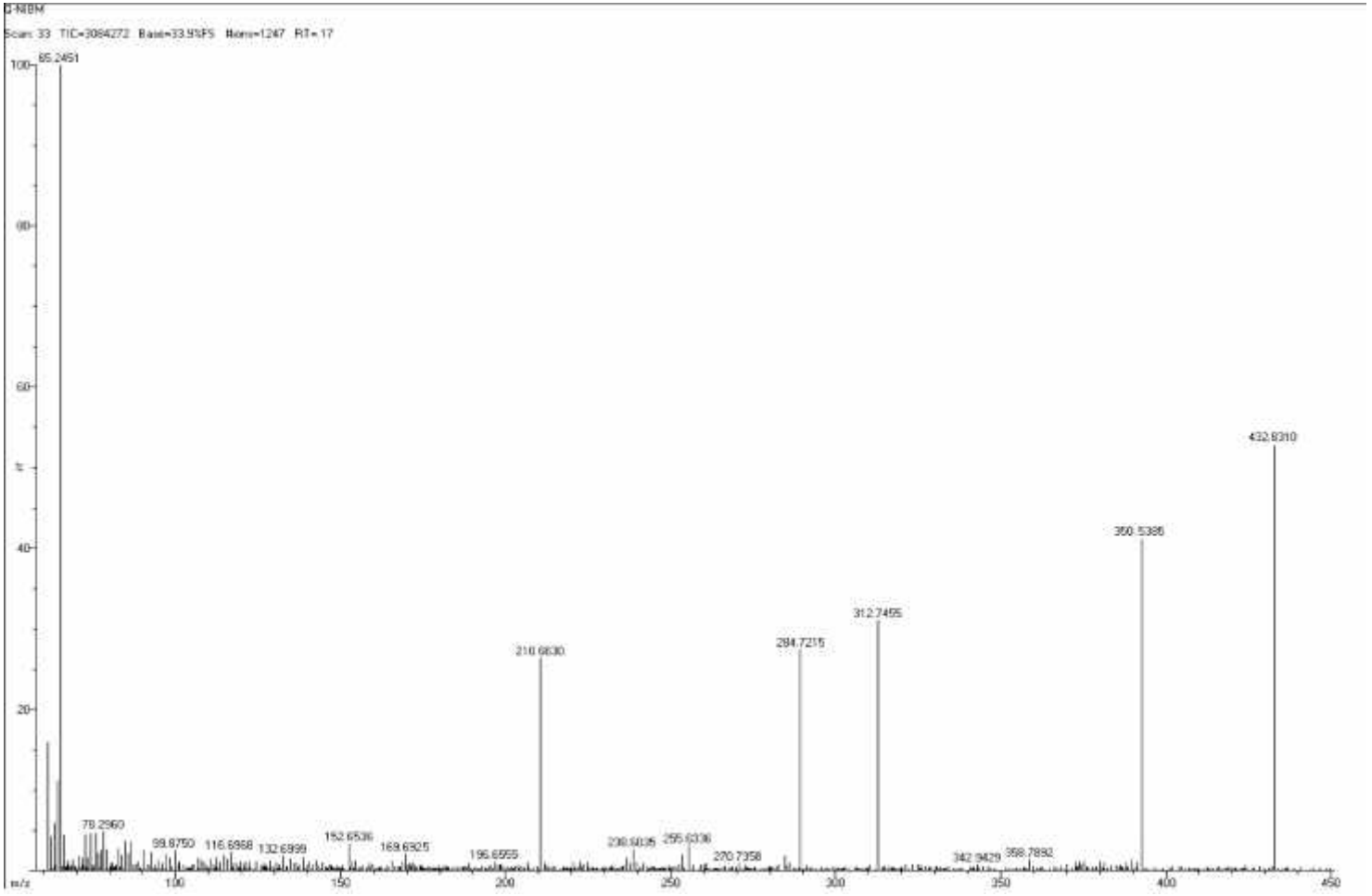
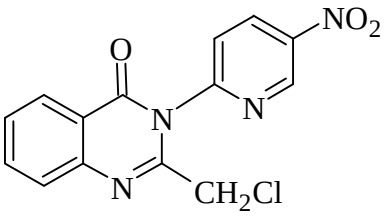


Figure 49: Mass Spectra of QNIBM

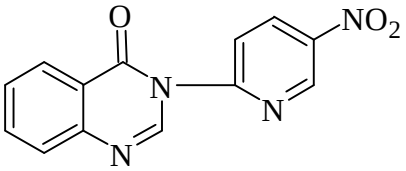
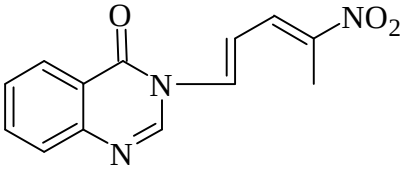
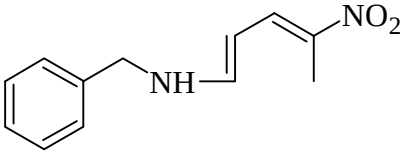
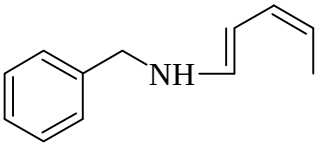
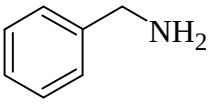
Interpretation of Mass spectra of QPIN



Molecular Weight: 316.45 Molecular Ion Peak: 316.59

The possible fragments of the molecule with the relevant to its m/z values are:

Table-30

S. No	m/z	Fragments
1	268.22	
2	257.24	
3	218.25	
4	173.25	
5	107.15	

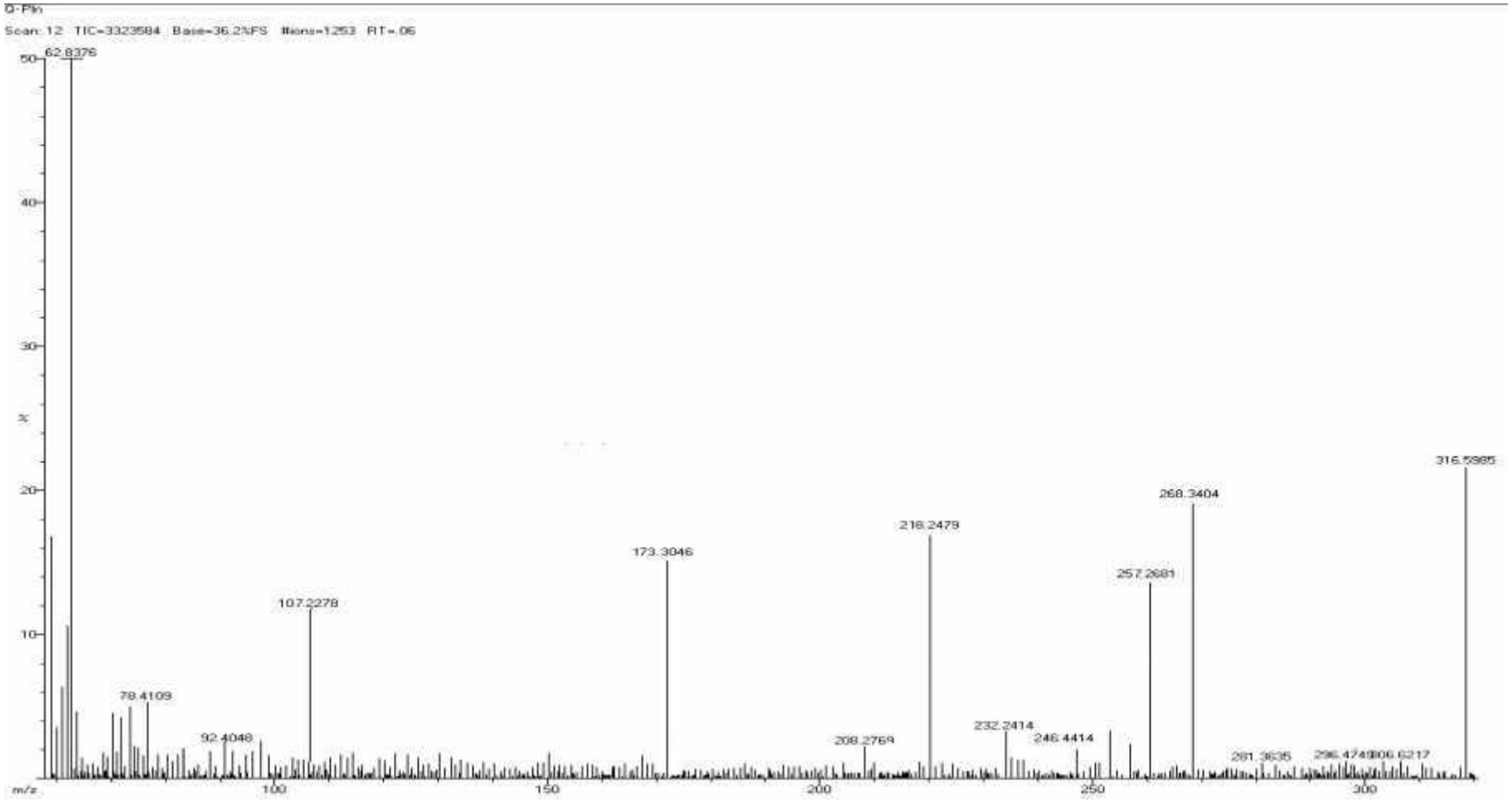
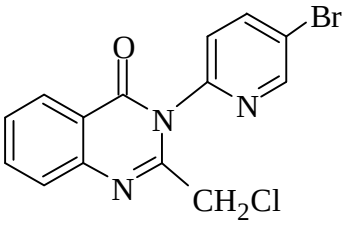


Figure 50: Mass Spectra of QPIN

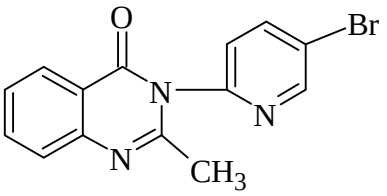
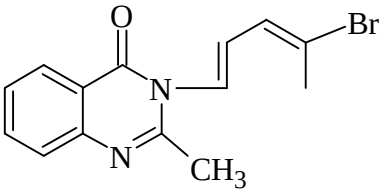
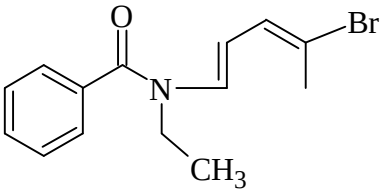
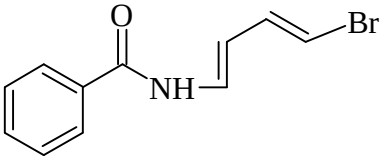
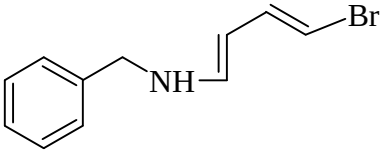
Interpretation of Mass spectra of QBrP



Molecular Weight: 350.57 Molecular Ion Peak: 350.59

The possible fragments of the molecule with the relevant to its m/z values are:

Table-31

S. No	m/z	Fragments
1	318	
2	306	
3	296	
4	253	
5	236	

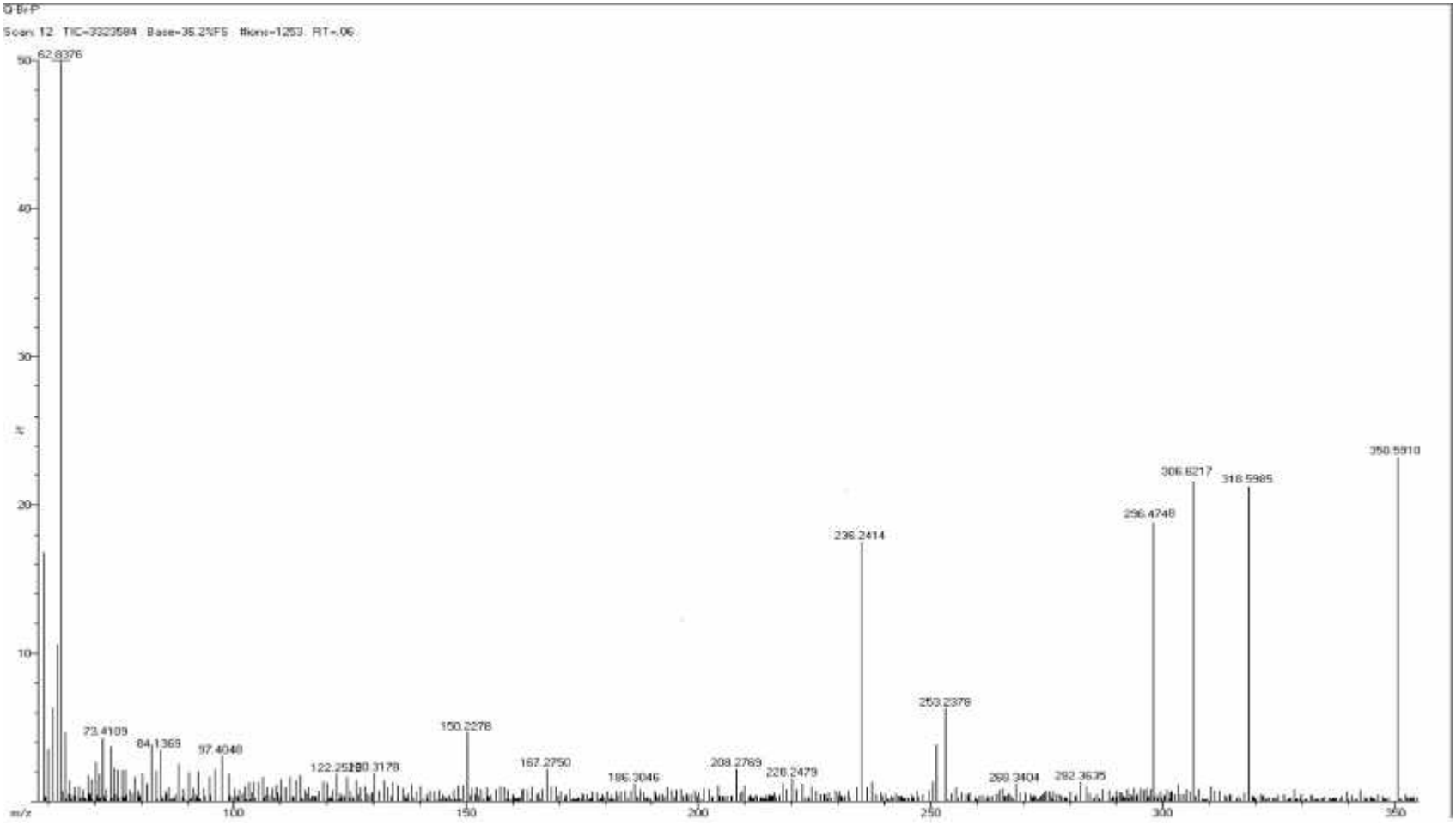


Figure 51: Mass Spectra of QBrP

Evaluation of analgesic activity

The analgesic activity of the synthesized compounds was evaluated by Eddy's hot plate method in mice with reference to the standard drug pentazocine.

Table-32. Results of Analgesic activity

Compound	Paw licking or jumping response (in sec)				
	0 min	30 mins	60 mins	90 mins	180 mins
Control	7.02 ±0.58	8.06 ±0.58	7.08 ±0.58	7.01 ±0.58	6.67 ±0.67
Standard (50mg/kg)	10.04±1.15	11.03 ±0.58	10.21 ±0.58	9.67 ±0.33	8.27 ±0.88
QAPA (50mg/kg)	7.02 ±0.78	9.76±0.32**	8.88±0.58**	8.78 ±0.78*	6.08±0.98
QIBM (50mg/kg)	8.06 ±0.58	9.33 ±0.33	9.73 ±0.33*	7.02±0.58	6.33 ±0.31

*P<0.05, **P<0.01. ***P<0.001 compare to 0 h response. One-way ANOVA followed by Bonferroni test.

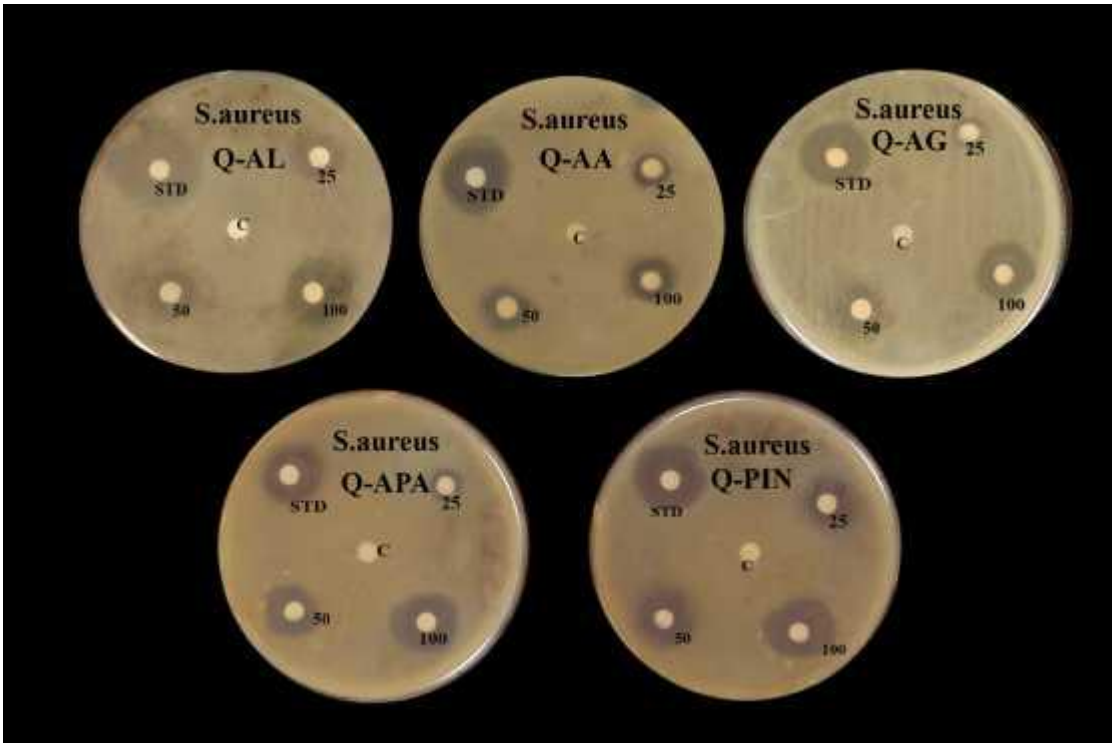
Screening of antimicrobial activity

i) Antibacterial Activity

The antibacterial activity of synthesized compounds were screened against both gram positive *(S. aureus)* and gram negative *(E. coli)* organism by agar disc diffusion method using ciprofloxacin as standard drug.

Table-33. Results of Antibacterial Activity

Compounds	Zone of Inhibition (in mm)					
	S. aureus			***E. coli***		
	25 (µg/ml)	**50 (µg/ml)**	**100 (µg/ml)**	**25 (µg/ml)**	**50 (µg/ml)**	**100 (µg/ml)**
QAL	22	26	31	11	14	20
QAA	19	22	26	19	22	24
QAPA	15	19	23	21	24	29
QAG	14	19	22	18	21	26
QBEN	15	18	27	20	23	29
QIBM	19	21	27	16	21	27
QNIBM	16	19	29	17	22	28
QPIN	22	26	28	20	22	28
QBrP	20	22	28	15	18	24
Ciprofloxacin 10(µg/ml)	39			38		



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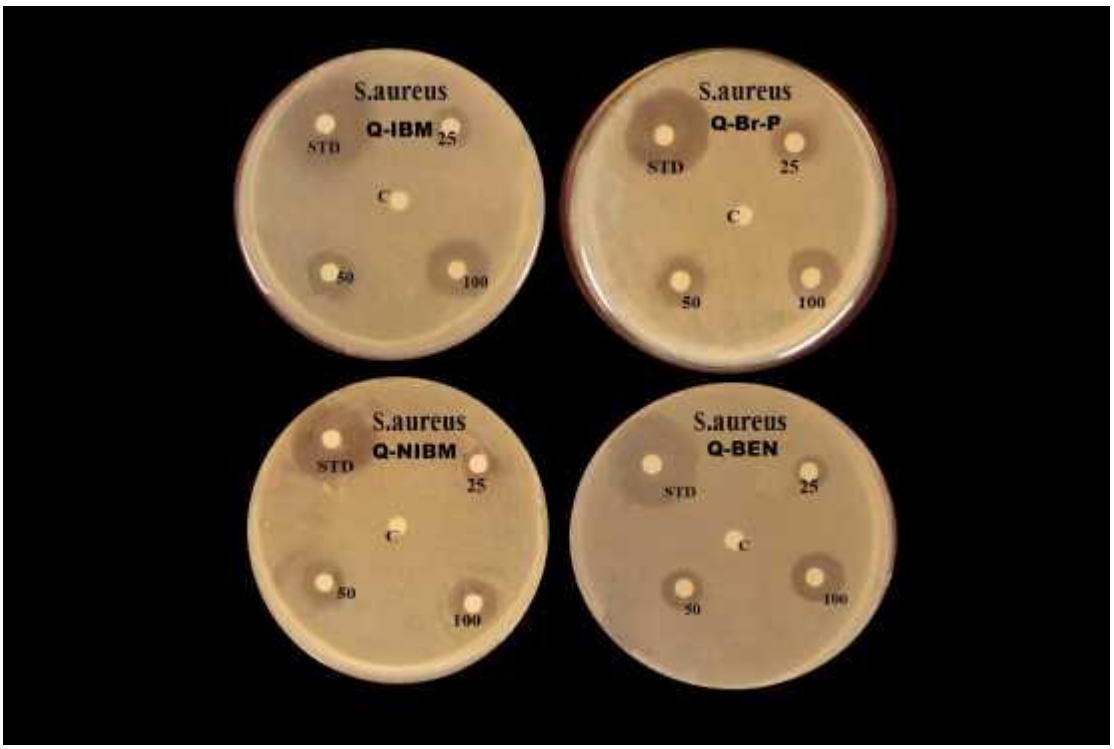
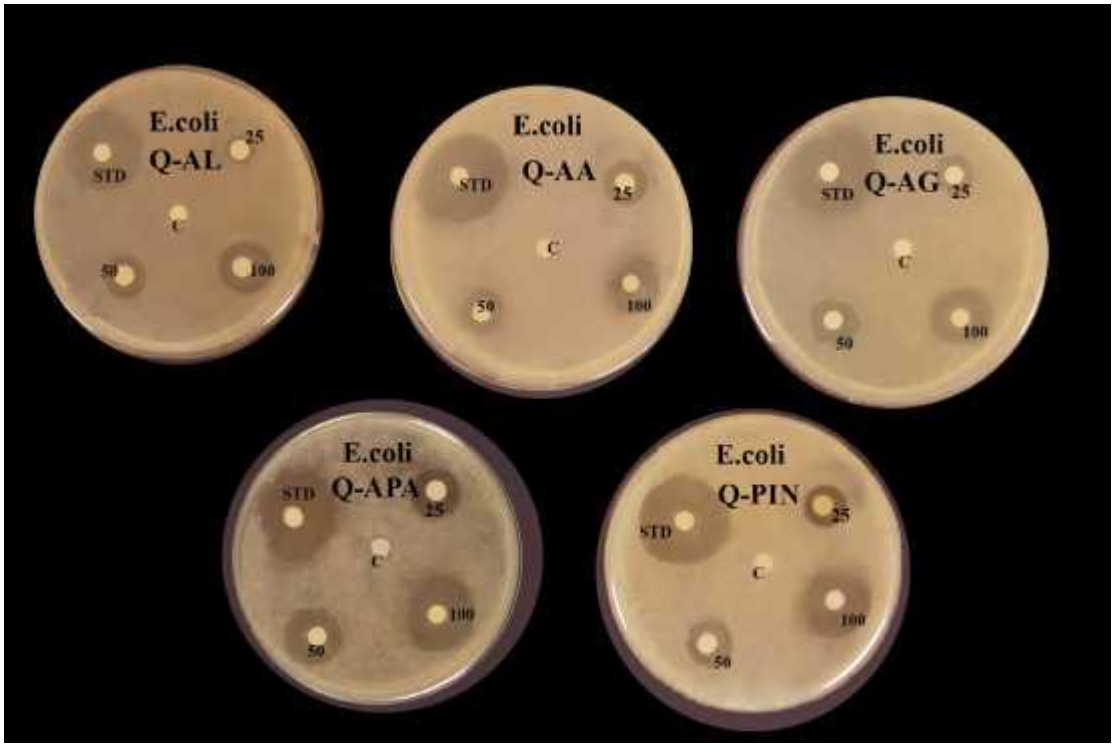


Figure 52: Antimicrobial activity of synthesized compounds against *S.aureus*



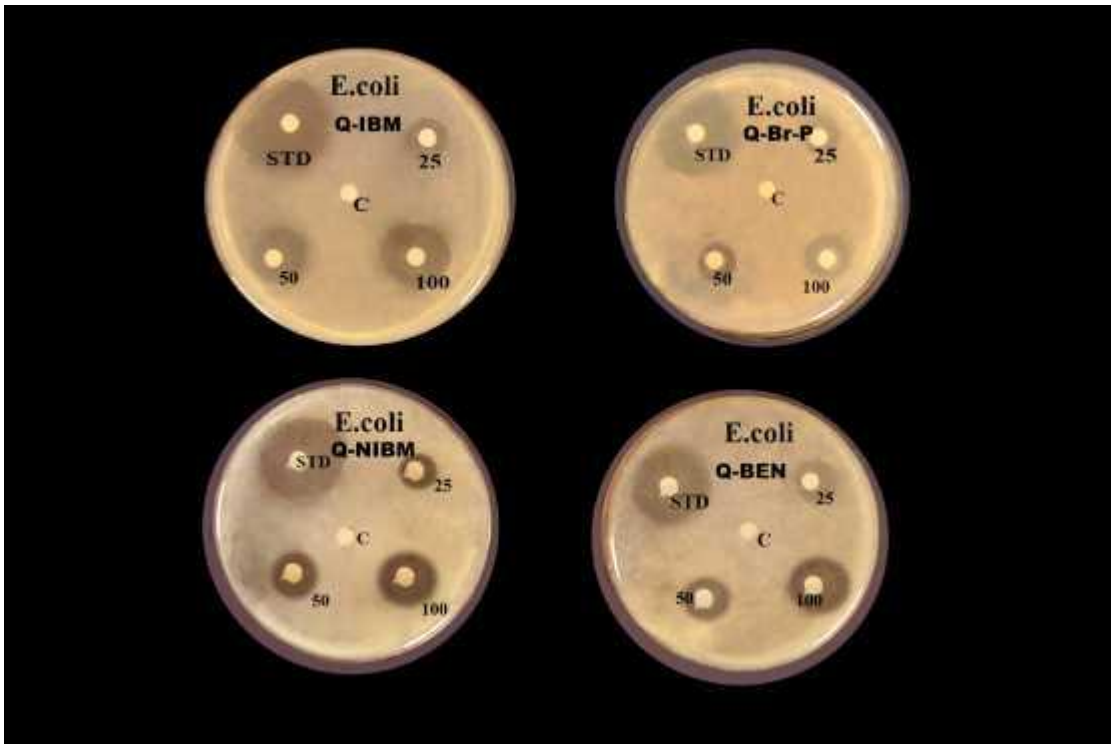


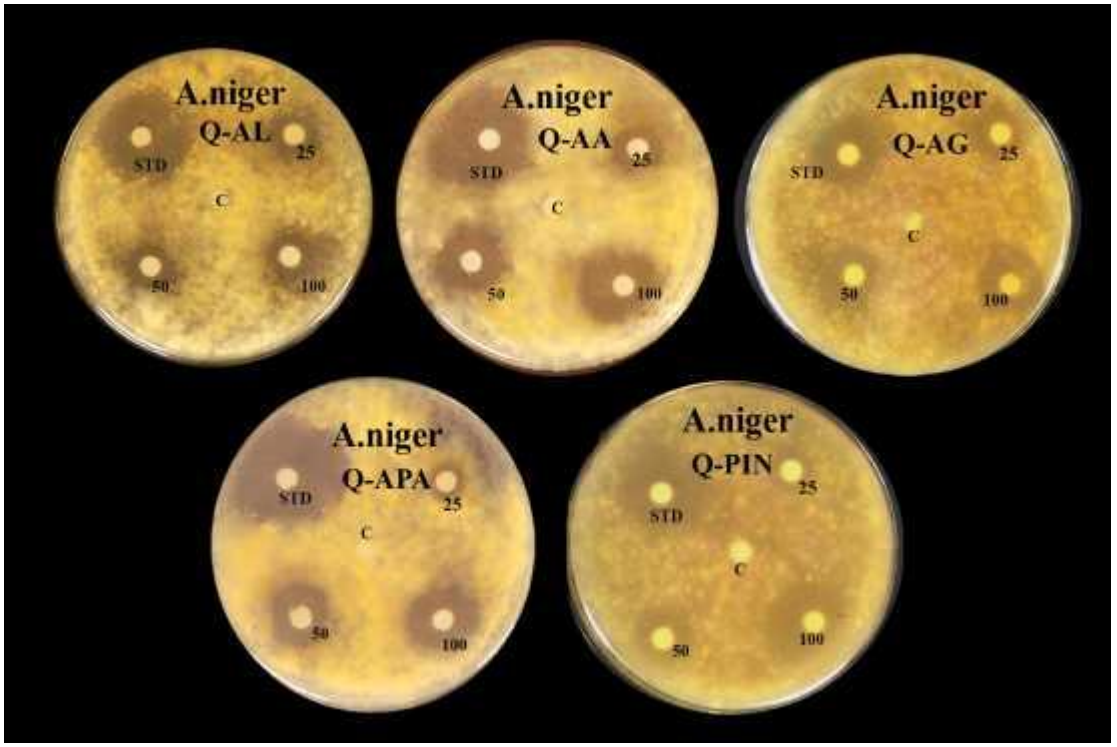
Figure 53: Antimicrobial activity of synthesized compounds against *E.coli*

ii) Antifungal Activity

The antifungal activities of synthesized compounds were screened against *A.niger* by agar disc diffusion method using ketoconazole as standard drug.

Table-34. Results of Antifungal activity

Compounds	Zone of Inhibition (in mm)		
	Aspergillus niger		
	25 (µg/ml)	**50 (µg/ml)**	**100 (µg/ml)**
QAL	22	26	31
QAA	19	22	26
QAPA	15	19	23
QAG	14	19	22
QBEN	15	19	23
QIBM	13	16	18
QNIBM	21	24	29
QPIN	22	26	28
QBrP	15	13	24
Ketoconazole 10(µg/ml)	39		



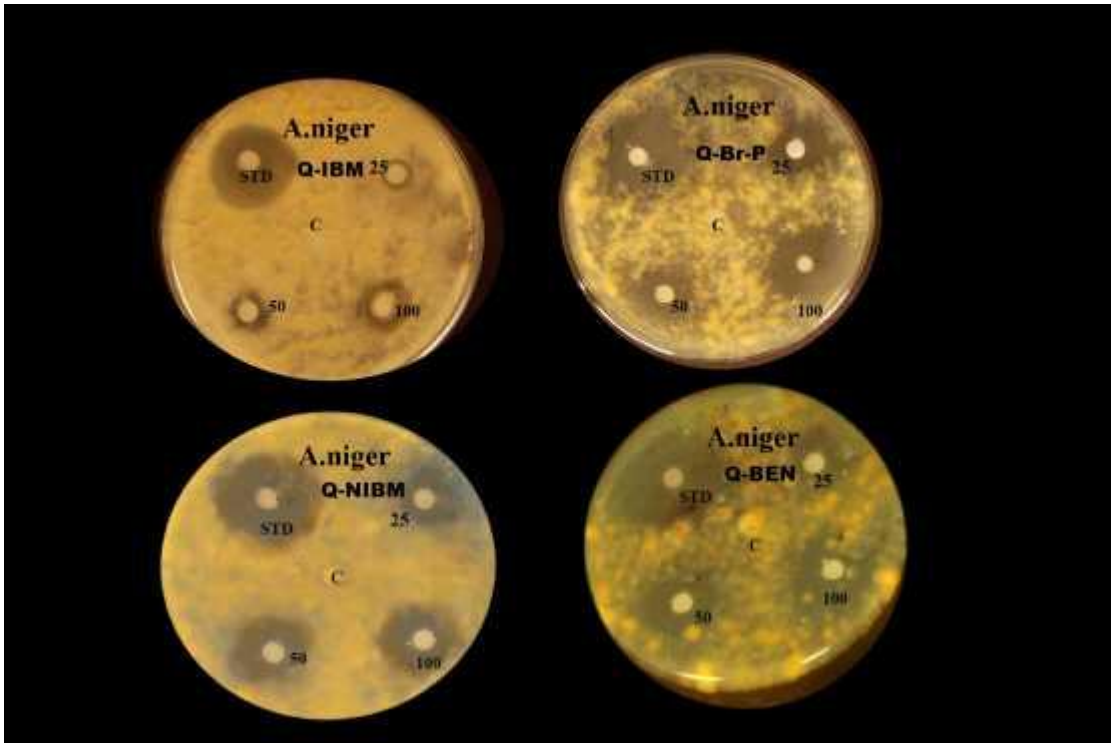


Figure 54: Antimicrobial activity of synthesized compounds against *A.niger*

DISCUSSION

Analgesic activity:

The synthesized compounds were tested for their Analgesic activity by Eddy's hot plate method where the source of pain is heat and the results of the compounds were compared with the standard drug pentazocine. The activity was observed up to 180 minutes.

The standard drug pentazocine showed increase in reaction time to the stimulus with maximum response at 60^{th} minute. Also at 60^{th} minute, both QAPA and QIBM were found to be significant compared to control. The activity was started to abolish gradually after 60^{th} min. One-way ANOVA followed by Bonferroni test was applied to the results to find the results are statistically significant or not in the used population.

Antimicrobial activity

The antimicrobial activity was screened disc diffusion method were Muller Hinton Agar media used for bacteria and Sabouraud's agar media used for fungal growth.

Antibacterial activity

Among the compounds used for antibacterial activity, QPIN, QNIBM, QBEN could show significant activity against both *(S. aureus)* and *(E. coli)* organisms. The QAL and QAA were somewhat selective for the inhibition of gram positive organism *(S. aureus).* The rest of the compounds could show only moderate activity at the optimal concentration of 100µg/ml when compared to 10µg/ml of ciprofloxacin as standard.

Antifungal activity

Among the compounds QAL, QNIBM and QPIN shown good antifungal activity against *A. niger* and rest of the compounds could show only moderate activity at the maximum concentration of 100µg/ml when compared to 10µg/ml of ketoconazole as standard.

SUMMARY

VI. SUMMARY

Totally nine compounds of newly modified quinazolin-4 (3H)-ones were synthesize Four derivatives of quinazolones were synthesized by incorporating the amino acids of Leucine, alanine, phenylalanine and glycine at third position of the ring. Anthranilic acid was treated with chloracetyl chloride and obtained N-chloro acetyl anthranilic acid. To synthesize amino acid incorporated quinazolones, the N-chloroacetyl anthranilic acid was treated with hydrazine hydrate and derived 3-amino-2-chloromethyl quinazolin-4(3*H*)-one. Then this intermediate was coupled with protected amino acids, using EDC as coupling agent. Finally the BOC was deprotected to have 2-chloromethyl-3-amino acid substituted quinazolin-4(3H)-ones.

Five derivatives of quinazolones were synthesize by incorporating the heterocyclic moieties of substituted Indole, Benzthiazole and Pyridine at third position of the ring. Anthranilic acid was treated with chloroacetyl chloride and obtained N-chloro acetyl anthranilic acid. To synthesize heterocyclic incorporated quinazolones, N-Chloroacetyl anthranilic acid was refluxed separately with 2-amino benzthiazole, 2- amino benzimidazole, 2-amino 5-nitro phenyl benzimidazole, 2-amino 5-nitro pyridine and 2-amino 5-bromo pyridine and resulting 2-chloromethyl-3-heterocycle substituted quinazolin-4(3*H*)-one.

The completion of the reaction was checked by TLC. The chloroform and petroleum ether mixture was used for the purification of the amino acid derivatives of quinazolinones and the heterocylic derivatives of quinazolinones was washed thoroughly with boiling water and recrystallized from acetone: ethanol mixture (1:1).

The structures of the synthesize compounds were confirmed with IR, ^{1}H NMR and Mass spectroscopy.

The synthesize compounds were tested for their analgesic activity by Eddy's hot plate method where the source of pain is heat and the results of the compounds were compared with the standard drug pentazocine. One-way ANOVA followed by Bonferroni test was applied to the results to find the results are statistically or not in the used population.

The synthesize compounds were screened for their antimicrobial activity for the gram positive, gram negative and fungal organism by disc diffusion method and the inhibition of microorganism were compared with the standard drugs ciprofloxacin and ketoconazole respectively.

The compounds QAPA, QIBM shown moderate analgesic activity.

The compounds QAL, QAA, QBEN, QNIBM and QPIN were shown significant activity against the organism used for the study.

CONCLUSION

VII. CONCLUSION

The two different set of quinazolones containing amino acids and heterocycles separately were synthesize and evaluated. The analgesic and antimicrobial activities are not very significant for one set of compounds than the other. Though all the compounds shown biological action it is better to incorporate the dipeptides and other heterocycles.

For the amino acid series the rationale cannot be drawn for the different amino acids used, since the aliphatic amino acid leucine and alanine activity is equal to aromatic amino acid phenyl alanine. There is no distinct difference in the biological action upon increasing the hydrophobicity through increasing number of carbons. May be the di or tripeptide incorporation at the 3rd position can give an idea about whether the amino acid/peptides incorporation in the quinazolones may overtake the heterocycles or not.

For heterocyclic incorporate series, among the Benzimidazole and Benzthiazole and pyridine used, the nitro group substituted compounds are active than others. May be the electron withdrawing group are required to enhance the biological action of the quinazolones.

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VIII.BIBLIOGRAPHY

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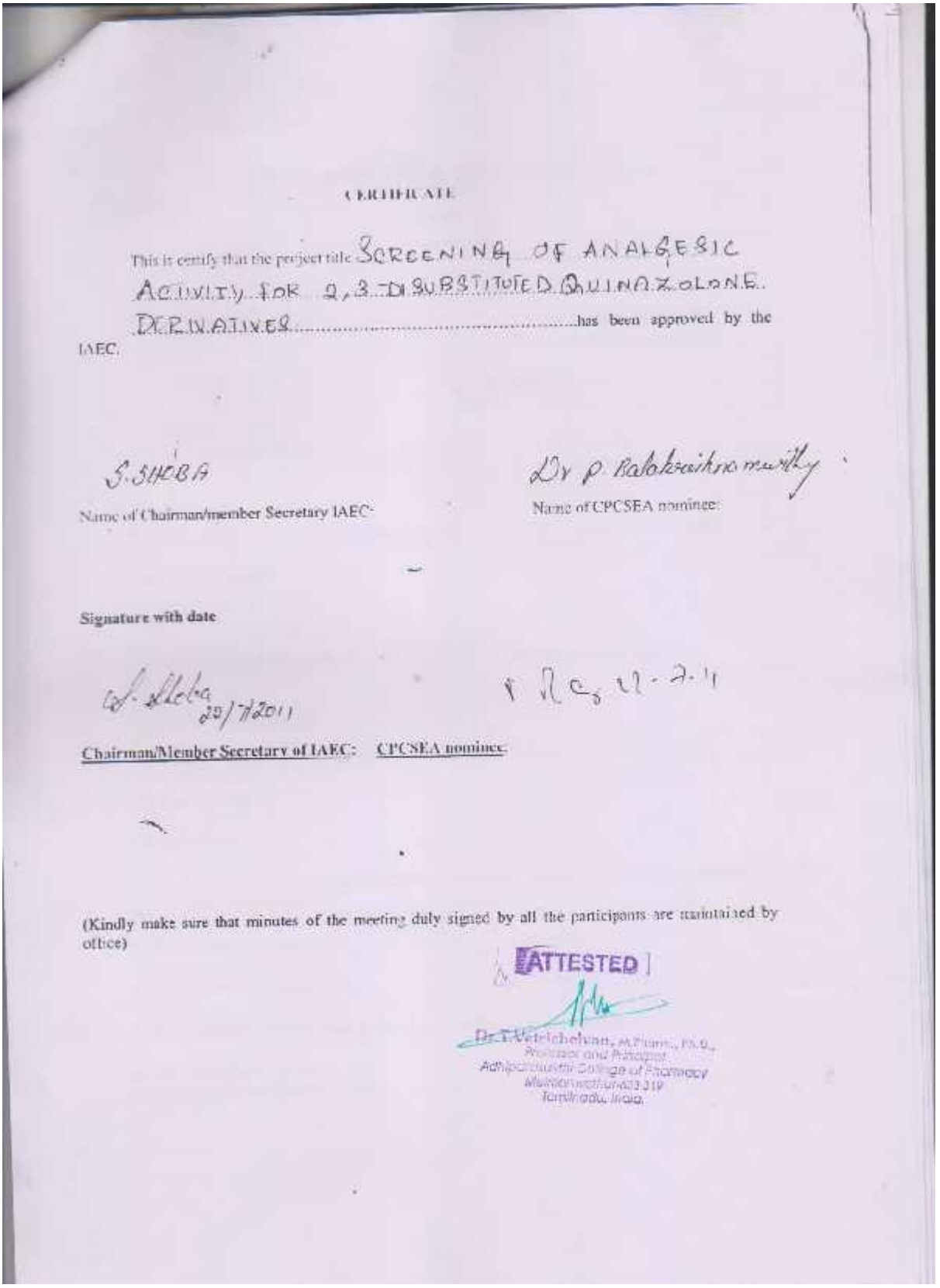
ANNEXURE

ANNEXURE I

The animal study protocol was approved by IAEC (Reg No: 409/01/CPCSEA).



CERTIFICATE

This is certify that the project title SCREENING OF ANALGESIC ACTIVITY FOR 2,3-DISUBSTITUTED QUINAZOLONE DERIVATIVES.....has been approved by the IAEC.

S. SHOBA

Name of Chairman/member Secretary IAEC

Dr P Balakrishnamurthy

Name of CPCSEA nominee

Signature with date

S. Shoba 22/7/2011

22-7-11

Chairman/Member Secretary of IAEC: CPCSEA nominee

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by office)

ATTESTED

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ANNEXURE II

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Research Article

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Synthesis of 2, 3-disubstituted quinazolone derivatives for analgesic and antimicrobial activities

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ABSTRACT

In view of the effective range of biological activities exhibited by quinazolinones, a series of 2, 3-disubstituted quinazolin-4-(3H)-ones have been synthesized and evaluated for analgesic, antibacterial and antifungal activities. Five derivatives of quinazolones were synthesized by incorporating the heterocyclic moieties of substituted Indole, Benzothiazole and Pyridine at third position of the ring. The structures of the compounds were confirmed on the basis of IR, ^{1}H NMR and Mass spectroscopy. The analgesic and antimicrobial activity of the compounds was screened by Eddy's hot plate method and Disc diffusion method respectively. All the five compounds could show analgesic and antimicrobial activities. The compound II showed better analgesic activity than other compounds. Significant activity was shown by compound IV against gram positive organisms and by compound I against gram negative organisms. The best antifungal activity was shown by compound IV.

Keywords: Quinazolones, Analgesic, Antibacterial and Antifungal activity.

INTRODUCTION

Quinazolin-4(3H)-one is a versatile heterocyclic moiety for the medicinal chemists for molecular modification due to its wide spectrum of biological activities. They have established a variety of biological activities like analgesic [1], anti-inflammatory [1], antihypertensive [2], antihistaminic, anticancer [3-4], sedative- hypnotic and antimicrobial activities [5-6]. The second and third positions are the most favourable positions for making substitutions on the quinazolone ring and many research works have evidenced this concept [7]. This prompted us to synthesize a new series of quinazolone derivatives by incorporating the heterocyclic rings at the third position. The 2-chloro methyl-3-heterocycle substituted quinazolones were synthesized by cyclizing the N-chloro acetyl anthranilic acid with amino substituted indole, benzthiazole and pyridine. The N-chloro acetyl anthranilic acid was prepared by acetylating the anthranilic acid. The structures of all the synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectroscopy. All the compounds have been screened for their analgesic, antibacterial and antifungal activities.

EXPERIMENTAL SECTION

The melting points were determined in open capillary tubes in a Hicon melting point apparatus. Infra-red spectrum was recorded in Jasco FT/IR 410 spectrometer by KBr pellet method and the ^{1}H NMR spectra was taken on a Bruker Avance II (400 MHz) NMR spectrometer using dimethylsulfoxide as solvent and chemical shifts are expressed in parts per million relative to tetra methyl silane as an internal standard. Mass spectra was recorded on shimadzu 2010A LC-MS. The completion of the reactions was checked by thin layer chromatography on silica gel G coated plates using chloroform: methanol (7:3) solvent system and the spots were detected by Iodine vapours. The animal study protocol was approved by Institutional Animal Ethical Committee. (Reg No: 409/01/CPCSEA).